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Role of Instream Rootwads as Juvenile Coho Salmon (*Oncorhynchus kisutch*) and Steelhead Trout (*O. mykiss*) Cover Habitat Under Varying Streamflows

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Coho salmon fry (*Oncorhynchus kisutch*) and steelhead parr (*O. mykiss*) occupied previously infrequently-used mid-channel areas of Kloiya Creek, British Columbia, Canada, once artificial rootwads were placed there. Ninety-nine percent of all coho salmon fry and 83% of steelhead parr occupied positions downstream of natural or artificial rootwads during artificially created drought, normal, and flood streamflows. Fish associated with rootwads regardless of distance from shore, but steelhead parr preferred rootwads away from shore while coho salmon fry preferred rootwads next to shore. Coho salmon fry increased their use of natural rootwads where currents were slow during floods, while steelhead parr increased their use of artificial and natural rootwads where light remained low during droughts. Young fish apparently selected areas having slower water 80% of the time because they provided shelter from adverse current, and areas having reduced light intensities 20% of the time because they provided protection from predators. Juvenile coho salmon and steelhead in Kloiya Creek selected locations with slower water velocities and reduced light intensities irrespective of the physical structure that caused them.

Lorsque des amas de racines artificiels y ont été installés, les alevins de saumon coho (*Oncorhynchus kieutch*) et les tacons de truite arc-en-ciel (*O. mykiss*) ont occupé des zones rarement utilisées du milieu du lit du ruisseau Kloiya en Colombie-Britannique au Canada. Quatre-vingt-dix-neuf pour cent de tous les alevins de saumon coho et 83 % des tacons de truite arc-en-ciel s'observaient en aval des amas de racines naturels ou artificiels au cours de sécheresses artificielles, d'écoulement normal et de crues. Les poissons recherchaient les amas de racines peu importe leur distance de la rive, mais les tacons de truite arc-en-ciel préféraient les amas éloignés de la rive, tandis que les alevins de saumon coho recherchaient les amas situés près des rives. Les alevins de saumon coho étaient plus portés à utiliser des amas de racines naturels, là où les courants étaient lents durant les crues, tandis que les tacons de truite arc-en-ciel ont augmenté leur utilisation des amas naturels et artificiels là où la lumière restait faible au cours des sécheresses. Les jeunes poissons semblent avoir préféré les régions à courant plus lent comme abri 80 % du temps, et les zones à intensité lumineuse réduite comme protection contre les prédateurs 20 % du temps. Les truites arc-en-ciel et les saumons cohos juvéniles du ruisseau Kloiya ont choisi des emplacements où la vitesse de l'eau était faible et l'intensité lumineuse basse peu importe s'il s'agissait d'amas de racines naturels ou artificiels.

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Windfall trees are important structural components creating and maintaining fish habitat in forested streams (Keller and Swanson 1979). The effects on fish populations of altering the amount of wood in streams can be varied. Fish populations can either increase or decrease following the removal of wood from streams (Murphy and Hall 1981). Consequently, confusion has developed over the function wood plays in the ecology of stream fish. To improve habitat conditions, some managers have added wood to streams (e.g. Seehorn 1985; Boehne and Wolfe 1986), while others have removed wood (Bryant 1983; Dolloff 1986; Stream Renovation Guidelines Committee 1983).

In the Pacific Northwest, trees in streams are often assumed to be providing "cover" habitat (Bisson et al. 1987). The American Fisheries Society defines cover as "anything that provides protection from predators or ameliorates adverse conditions of streamflow and/or seasonal changes in metabolic costs" (Habitat Inventory Committee 1986). In streams the rootmass of windfall trees may function to provide both shelter and protection, but the proportion of shelter versus protection may decline as streamflow decreases. Obviously rootwads in streams are not providing shelter from current during low-streamflow periods normal in late summer. Consequently the function, and therefore possibly the importance, of rootwads in streams varies seasonally with streamflow. Furthermore, in coastal Pacific salmon streams fish often distribute themselves along the stream edges downstream from rootwads rather than near the center of the stream (Lister and Genoe 1970). Therefore the location of the rootwad relative to the stream edge may also affect its role as habitat in the ecology of stream fish.

The purpose of this study was to investigate the effect of the abundance and position of rootwads on their function as "cover" habitat in the ecology of young salmonids in streams, especially in relation to variable streamflows. Specific hypotheses were: (1) juvenile salmonids use positions downstream from rootwads for shelter from current at all streamflows, not for protection from predators, (2) positions downstream from rootwads will be occupied if the rootwad is next

to shore but not if the rootwad is in midstream, and (3) juvenile salmonids are attracted to rootwads themselves and not just the conditions caused by rootwads.

Methods

Study Area

The study was conducted in Kloiya Creek, British Columbia, a small coastal stream 12 km southeast of Prince Rupert. The watershed is 108 km^2 and has a wet coastal climate receiving an average of 2855 mm of rainfall annually. Streamflow in Kloiya Creek is controlled by a dam 3.2 km upstream from the Pacific ocean. Seventy-seven percent of the streamflow (6.4 $m^3 \cdot s^{-1}$) drains through Kloiya Creek, but the rest (1.9 $m^3 \cdot s^{-1}$ or 23%) is taken out at the dam through a gravity fed pipeline to a local pulp mill. Streamflow can be manipulated by storing or spilling water from the reservoir (Bravender and Shirvell 1989).

Kloiya Creek has populations of at least five anadromous fish species. Average annual escapements into the Kloiya Creek system include 3000 sockeye salmon (*O. nerka*), 200 chinook salmon (*O. tshawytscha*), 500 pink salmon (*O. gorbuscha*), 700 coho salmon (*O. kisutch*), and 250 steelhead (*O. mykiss* formerly *Salmo gairdneri*). All of the chinook salmon and pink salmon and about 30% of the coho salmon and steelhead spawn in Kloiya Creek, while all of the sockeye salmon and about 70% of the coho salmon and steelhead spawn above Kloiya dam in the reservoir tributaries. In addition, there are cutthroat trout (*Salmo clarki*) and Dolly Varden (*Salvelinus malma*) populations of unknown size; these may be anadromous (Hancock et al. 1983).

Experimental Design

Natural and artificial rootwads

Because densities of juvenile Pacific salmon are related to the complexity of their habitat (Murphy et al. 1985), and because the rootmass of trees is the most complex single wood piece naturally occurring in salmon streams, I chose rootwads as the unit of wood for this experiment. Salmonid abundance was chosen as the response variable. To investigate the response of fish distribution to rootwads, a single pool was chosen which had a natural rootwad at one side, and an adjacent area of similar depth, velocity, and cobble substrate but no rootwads. This location, therefore, represented similar habitat, with and without rootwads. To investigate whether fish would use rootwads in the center of the stream, an artificial rootwad was placed 3 m laterally to the natural rootwad. This created a pattern of natural rootwad, no rootwad, and artificial rootwad, all other aspects of the habitat being similar. To investigate whether the usefulness of rootwads as habitat depends upon their location relative to the shore, two additional artificial rootwads were placed laterally to the first at increasing distances from the shore. This created a pattern of natural rootwad and artificial rootwads at 1/4, 1/2, and 3/4 of the stream width across the stream with areas of similar conditions but without rootwads between each (Fig. 1). The thalweg (Morisawa 1968) at the study site came close to the natural rootwad, consequently the three artificial rootwads were positioned along a gradient of depth and velocity. This positioning of the artificial rootwads created a "wood" treatment of three levels of wood from which the fish populations could choose: (1) natural rootwad, (2) no rootwad, or (3) artificial rootwads at three distances from shore for a total of five in-stream "positions."

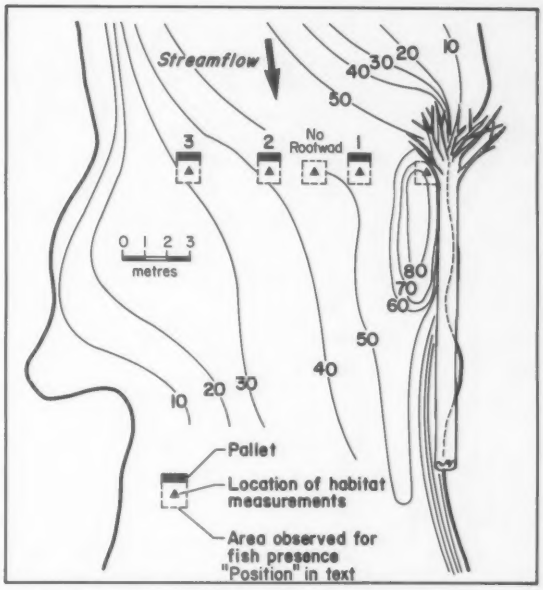

FIG. 1. Map of the study area within Kloiya Creek showing natural, artificial, and "no" rootwad locations, plus water depth contours (cm) at the drought streamflow level. Map drawn to scale.

The artificial rootwads were intended to simulate natural rootwads and consisted of standard 100 × 120 × 13 cm warehouse pallets with alternating slats on two sides. The unaligned slats thus created spaces betweeen the two sides of the pallet similar to the interstitial spaces between individual roots of a natural rootwad. The pallets were oriented perpendicularly to the stream flow with the slats vertical and the tops slanted downstream at 115 degrees with two wooden legs creating an "A-frame" profile. They were stained black and were held in position with sand bags placed on their tops. The orientation of the artificial rootwads mimicked the natural rootwad, which was approximately 3 m in diameter and lay on its side, although only one half of its mass was wetted at most stream flows. Some fish occupied positions underneath the trunk of the natural rootwad so this position was included as part of the area chosen for fish and habitat sampling at the natural rootwad (see Fig. 1). Because the pallets were inclined downstream, they also overhung the areas observed for fish and habitat sampling at the artificial rootwad positions. The artificial rootwads were installed on June 14 and allowed to stabilize for 10 d before observation began; fish, however, began to occupy positions behind them within 3 h of their placement.

Effect of rootwads on habitat conditions

For the experiment to be a valid test of the influence of rootwads away from the stream margin, it was first necessary to ensure that the artificial rootwads effectively simulated the natural rootwad. Conditions downstream from the artificial rootwads were compared with conditions downstream from the natural rootwad and at the "no" rootwad location. The depths, velocities, and light intensities for the five positions over all streamflow levels are shown in Fig. 2. Over all streamflow levels, mean water velocities and light intensities downstream

STREAMFLOW		Natural Rootwad	Artificial Rootwad[1]	No Rootwad	Mean
	Drought	67.5 1.5 3.7 8.6	48.1 6.8 2.6 6.0	40.8 31.7 7.5 17.4	50.5 10.7 3.8 7.0
	Normal	76.7 15.3 3.7 4.7	57.0 13.8 2.9 3.7	52.0 40.3 7.5 9.5	59.9 19.4 4.0 10.2
	Flood	86.3 16.3 3.3 3.3	64.9 16.9 3.4 3.4	60.3 46.0 11.5 11.5	68.5 22.6 5.0 11.5
	Mean	74.5 8.7 3.6 5.4	54.5 11.1 2.9 6.1	48.5 37.4 8.4 21.6	57.3 25.0 4.1 9.1

STREAMFLOW		Artificial Rootwad 1	Artificial Rootwad 2	Artificial Rootwad 3
	Drought	63.0 7.5 2.4 5.6	45.5 8.5 2.5 5.8	35.7 4.3 3.0 6.7
	Normal	73.0 20.0 3.2 4.0	53.3 10.7 2.8 3.5	44.7 10.7 2.5 3.2
	Flood	80.7 26.0 4.0 4.0	62.3 15.0 3.1 3.1	51.7 9.7 3.1 3.1
	Mean	69.9 15.3 3.0 5.8	51.7 10.7 2.7 5.8	41.9 7.3 2.8 6.7

[1] The mean of artificial rootwads 1, 2 and 3.

KEY

D V	D = water depth (cm), V = water velocity ($cm \cdot s^{-1}$)
L SL	L = light intensity ($lux \cdot 10^3$), SL = standardized light intensity (L X 1.0 for flood, L X 1.26 for normal, L X 2.32 for drought)

FIG. 2. Mean habitat conditions available at three levels of streamflow and three categories of wood (i.e. natural, artificial, and absent).

of the artificial rootwads were not significantly different from those that existed downstream from the natural rootwad (DMRT, $P > 0.05$, Fig. 2). Conditions downstream from both the natural and artificial rootwads were significantly slower and darker (DMRT, $P < 0.05$) than the position without a rootwad (Fig. 2). The mean depth downstream from the three artificial rootwads was significantly less than the location downstream from the natural rootwad (DMRT, $P < 0.05$, Fig. 2). This was due to the sloping nature of the streambed at the study site where the thalweg was adjacent to the natural rootwad (Fig. 1). However, the depth downstream from artificial rootwad 1 was not significantly different from that downstream from the natural rootwad (DMRT, $P > 0.05$, Fig. 2). Artificial rootwad 1 therefore simulated the natural rootwad for all the variables considered in this study, while artificial rootwads 2 and 3 simulated the natural rootwad except for water depth.

Streamflow

To investigate the response of salmonids to rootwads under different streamflows, streamflow in the stream was experimentally altered to create "drought", "normal", and "flood" conditions. The experiment was conducted during June and July

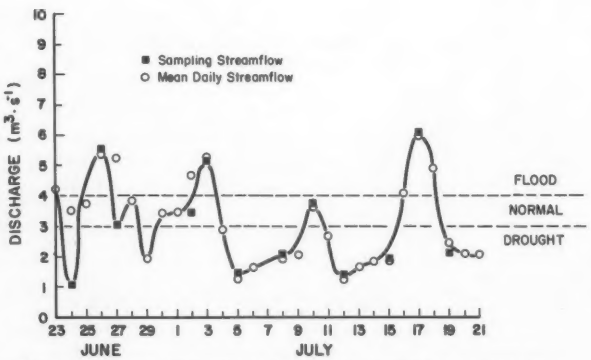

FIG. 3. Mean daily streamflow in Kloiya Creek, British Columbia from June 23 to July 21, 1985.

1985. The mean streamflow during June and July 1985 was 3.68 $m^3 \cdot s^{-1}$. The mean streamflow during June and July each year from 1975 to 1984 was 3.41 $m^3 \cdot s^{-1}$. Streamflows between 3.0 and 4.0 $m^3 \cdot s^{-1}$ were therefore classified as "nor-

mal", streamflows <3.0 $m^3 \cdot s^{-1}$ as "droughts", and streamflows >4.0 $m^3 \cdot s^{-1}$ as "floods." Streamflow was altered to create alternating drought, normal, and flood conditions (Fig. 3). Six samples were taken during droughts, three during normal streamflows, and three during floods, giving a total of 12 samples at the three streamflow levels within a period of 28 d. There were no replicates in space, only replicates in time. This allows a valid assessment of rootwads provided the rootwad and "no-rootwad" positions are sampled simultaneously (Stewart-Oaten et al. 1986).

Effect of streamflow on habitat conditions

Because rootwads are commonly thought to provide an important "shelter" function to fish during high streamflows by deflecting and reducing current (Bisson et al. 1987), habitat conditions caused by the drought, normal, and flood streamflows were compared to determine whether the streamflow levels created significantly different conditions. In general, lower streamflows caused conditions at all locations to be shallower, slower, and brighter; increased streamflows caused conditions to be deeper, faster, and darker (Fig. 4). When all five positions were considered together, the drought streamflows created significantly slower (DMRT, $P<0.05$) and shallower conditions (DMRT, $P<0.05$) than normal streamflows at all locations. But, while conditions created by flood flows were deeper and faster than at normal streamflows (Fig. 2), the differences were not large enough to be significant (DMRT, $P>0.05$).

Relative light intensities were not significantly different between streamflow levels; however, there was a trend for light intensities underwater to be higher during drought flows and lower during flood flows. This appeared to have been the result of higher turbidities caused by higher streamflows.

The streamflow and wood treatments combined were intended to create the range of habitat conditions juvenile Pacific salmonids might normally encounter in natural streams during spring. The treatments created gradients of water depth, water velocity, and light intensity along which fish could choose positions which most suited them. By arranging the physical conditions at the study site as was done it was possible to cause the gradients of light intensity and water velocity to be perpendicular to each other (see Fig. 4). This meant light intensity and water velocity could not be maximum at the same time. If shelter is related to water velocity and protection is related to light intensity, this separated the shelter function from the protection function of rootwads, and compelled the fish to chose one or the other at different streamflows.

Measurement of Fish and Habitat Conditions

The response of the fish to the available habitat conditions was assessed by observing their distribution among the five positions at 12 streamflows. A diver using a snorkel identified the species present, counted them, and estimated their lengths at each of the five positions for each streamflow. The observer cautiously entered the water downstream, and with minimal disturbance approached each position and enumerated fish in a 1 m^2 area immediately downstream of the rootwads. Estimates of fish length were confirmed later by downstream trapping and beach seining (Table 1). Following counts of fish at each position, water depth, water velocity, and light intensity in the center of the observation area were measured. Preliminary observations determined that juvenile chinook salmon and coho salmon in the study area were occupying positions approximately 10 cm above the stream bottom ($\bar{x}=9.2$ cm, $n=180$). Water velocity, therefore, was measured at 10 cm above the bottom, and light intensity was measured both at 10 cm above the bottom and at the water's surface. Light intensity was measured with a Li-Cor integrating quantum photometer equipped with an underwater photometric sensor.

Cloud cover varied on different sampling dates. The light intensity measurements at the unshaded surface of the "no-rootwad" position (see Fig. 1) were taken as a measure of the brightness on each sampling date. The mean light intensity at this position for all samples at each streamflow level was then taken as an index of the relative brightness which occurred during each streamflow level. The brightest series of days occurred during the flood streamflows, the next brightest days occurred

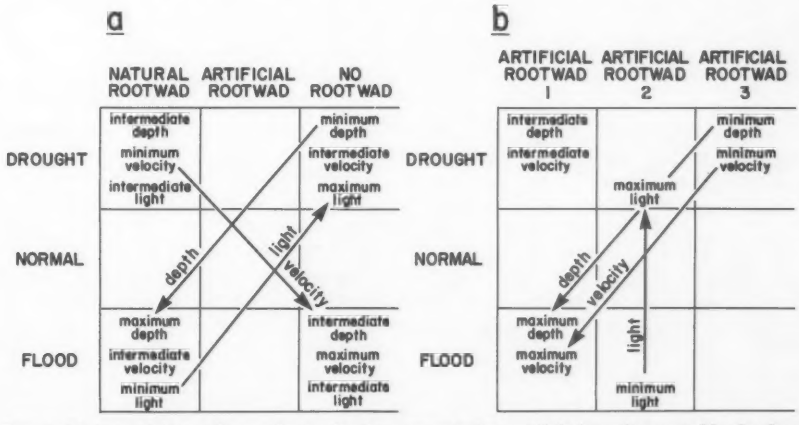

FIG. 4. (a) Axes of the gradients of water depth, water velocity, and light intensity created by the three levels of rootwads and streamflow in the experiment. (b) Gradients of the physical variables among the three artificial rootwads. Note that panel b comprises the middle column of panel a. Since light intensity was not significantly different downstream of either of the artificial rootwads at any streamflow, panel b tests for selection of only two variables (depth and velocity) while panel a tests selection for three variables.

TABLE 1. Fish community of Kloiya Creek during June–July 1985 at the natural, artificial, and no-rootwad locations and the average habitat conditions it used during drought, normal, and flood streamflows. Standard deviations are in square brackets.

Group	Total number of fish	Average number of fish observed per m^2[a]	Estimated mean length (mm)	Measured mean length (mm, *N*)	Mean water depth occupied (cm)	Mean water velocity occupied ($cm \cdot s^{-1}$)	Mean light intensity occupied[b] ($lux \cdot 10^3$)
Coho salmon fry	256	4.3	50	48.7 (128) [5.5]	61.2 [17.2]	7.7 [8.5]	3.0 [1.2]
Steelhead parr	122	2.0	124	105.3 (109) [17.6]	56.5 [13.4]	12.8 [12.0]	2.8 [1.6]
Dolly Varden	30	0.5	225		77.1 [11.3]	12.0 [11.0]	3.5 [2.0]
Steelhead fry	20	0.3	34	33.2 (240) [2.5]	38.1 [4.3]	5.2 [7.6]	3.1 [0.6]
Sockeye salmon adults	17	0.3	Undetermined		69.7 [7.8]	17.0 [6.4]	2.8 [1.0]
Chinook salmon fry[c]	8	0.1	53	54.2 (108) [6.4]			
Coho salmon smolts[c]	8	0.1	116	94.4 (23) [8.9]			
Cutthroat trout adults[c]	1	0.0	125				
	460	7.7					

[a]i.e. coho salmon fry mean abundance during experiment for all five locations = 4.3 per m^2.

[b]For comparison the mean unshaded light intensity (i.e. not downstream from a rootwad) available 10 cm above the stream bottom was 8.4×10^3 lux (SD = 5.90).

[c]Abundance of these groups in the experiment was so low that the habitat conditions they used are unreliable and are not reported.

during the normal streamflows, and the darkest days occurred during the drought streamflows (see Fig. 2). So that the effect of shading caused by the rootwads or change in brightness caused by different streamflows could be assessed, the light measurements taken during the normal streamflows were multiplied by 1.26 and the light measurements taken during the drought streamflows were multiplied by 2.32. These numbers were the ratios between the mean light intensity at the surface of the "no-rootwad" position during flood streamflows and the mean light intensity at the same location during the normal streamflows or the drought streamflows, respectively. This correction standardized all light measurements as if they had been taken on days of equal brightness. Differences in light intensities at the five positions could than be attributed to either the effect of cover or the effect of higher or lower streamflows and not be confused as an effect due to different day brightness (see Fig. 2).

Prior to the experiment, continuous observations of fish during a change of streamflow showed that fish reacted almost instantaneously by redistributing themselves first vertically then laterally. In all cases except the first, third, and fourth samples, the streamflow at which observations were made was not greatly different from the mean daily streamflow on that date (Fig. 3). For observations on the first, third, and fourth sample dates, the fish had less time to reposition themselves after a change in streamflow before observations began than on the other sample dates. However, because their response to changes in streamflow was so rapid, I judged this shorter adjustment period to have had no effect on the positions and habitat conditions they would have ultimately occupied. In any case, no observations or measurements were taken within 1 h following a streamflow change. All observations of fish abundance, and measurement of habitat conditions, were made between 0900 and 1500 h. The experiment was conducted from June 24 to July 25, 1985. Streamflows prior to the experiment (June 14–23) averaged 6.0 $m^3 \cdot s^{-1}$ (maximum = 9.0 $m^3 \cdot s^{-1}$, minimum = 3.3 $m^3 \cdot s^{-1}$).

Statistical Analysis

In the spring, when this experiment was conducted, the fish community in Kloiya Creek was changing rapidly because adult sockeye salmon, Dolly Varden trout, and cutthroat trout were migrating upstream, chinook salmon smolts and coho salmon smolts and fry were migrating downstream, and steelhead trout fry were emerging from the gravel. This caused the total abundance of fish at the study location to differ on each sampling date. To analyze the choices of each species for the different amounts of cover between dates, I calculated the proportion of the total of each species observed on each date that occupied each of the five positions. Each replicate in time, therefore, was not conducted with the same group of fish, but with a blended group consisting of naive (new) fish and "experienced" fish.

To assess whether the three levels of wood (natural rootwad, artificial rootwad, and no rootwad), and the three levels of streamflow (drought, normal, and flood), created distinct differences in the depth, velocity, and light intensity at each of the five positions, analysis of variance with Duncan's multiple range tests (DMRT) were used to compare the overall means of the habitat variables for each treatment level. Differences in the proportion of each species at the five positions at each of the streamflow levels were tested for significance with Student's *t*-tests. Change in the habitat variables for each of the five positions between the streamflow levels was tested for significance with *t*-tests. The relative importance of depth, velocity, and light intensity in influencing selection of positions by the fish was determined by building multiple linear regression models. The criterion used to judge the best model was r^2, the coefficient of determination. Stepwise forward, backward, and

maximum r_2 improvement techniques were used to build the best regression model. All statistical analyses were conducted using SAS systems software (SAS Institute Inc. 1985).

Results

Habitat Preferences

In 12 observations (six at drought flows, three at normal flows, and three at flood flows × five positions) 460 fish of seven species were counted (Table 1). Coho salmon fry and steelhead trout parr were the most abundant and made up over 80% of the observations. Chinook salmon fry, coho salmon smolts, and adult cutthroat trout were rarely observed. No individuals of any species were ever observed within the interstitial spaces of either the natural or artificial rootwads. All fish were in open water either downstream or underneath parts of the rootwads. When they were present, juvenile chinook salmon and adult cutthroat trout always occupied positions downstream of artificial rootwads 1 and 3 in the middle of the river, while coho salmon smolts always occupied the position downstream from the natural rootwad next to shore. Because these age classes of these three species were so infrequently observed, analysis of their habitat use is not reported.

When adult Dolly Varden and sockeye salmon were present, they used positions only behind either the natural rootwad or artificial rootwad 1, regardless of the sreamflow. These two positions straddled the thalweg, consequently these species may have been selecting positions more on the basis of depth than on the presence or absence of rootwads. When present together, individuals of these two species were always close to each other, and because of their short duration at the study site, they may have been migrating upstream together.

Two hundred and fifty six coho salmon fry were observed: 165 under drought streamflow ($\bar{x}$ = 28 per sample, SD = 11), 55 under normal streamflow ($\bar{x}$ = 18 per sample, SD = 10), and 36 under flood streamflow ($\bar{x}$ = 12 per sample, SD = 4). Ninety-nine percent of all the coho fry observed occupied positions downstream of either the natural or the artificial rootwads (Fig. 5). This affinity of coho fry for rootwads occurred regardless of streamflow. They showed no reluctance to occupy locations downstream from rootwads away from the stream margin.

Most coho salmon fry occupied positions downstream from the natural rootwad. This location was always the deepest, and it was the slowest, and darkest except for positions downstream from artificial rootwads 2 and 3 during the normal and flood streamflows. When coho fry did use positions downstream of the artificial rootwads, they most frequently used artificial rootwad 3, where water was shallowest and slowest, and they least frequently used the position downstream from artificial rootwad 2 which had intermediate water depth and velocity (Fig. 2). Because the natural rootwad and artificial rootwad 3 were respectively the deepest and shallowest positions, coho fry were aparently not selecting positions on the basis of water depth, but were simply accepting depths that occurred with their preferred level of some other variable.

Steelhead parr were the second most abundant fish group present (Table 1). One hundred and twenty were observed: 71 under drought streamflow ($\bar{x}$ = 12 per sample, SD = 5), 30 under normal streamflow ($\bar{x}$ = 10 per sample, SD = 9), and 19 under flood streamflow ($\bar{x}$ = 7 per sample, SD = 1). Their use of habitat downstream from rootwads contrasted sharply with that of the coho salmon fry. Unlike coho fry, steelhead parr used the natural rootwad position the least, and, on average, they most frequently chose positions downstream from the artificial rootwads (Fig. 6). Their use of positions downstream of the different artificial rootwads was about equal and they showed no overall preference for any individual artificial rootwad. Although approximately 80% of the steelhead parr used positions associated with rootwads, 20% used the position with no rootwad. This location was significantly faster and brighter than the other locations in the experiment (Fig. 2).

Fish Movements

Coho salmon fry showed a strong affinity for conditions downstream of rootwads at all streamflows. There was no significant redistribution when streamflows decreased from normal to drought streamflows. However, when streamflows

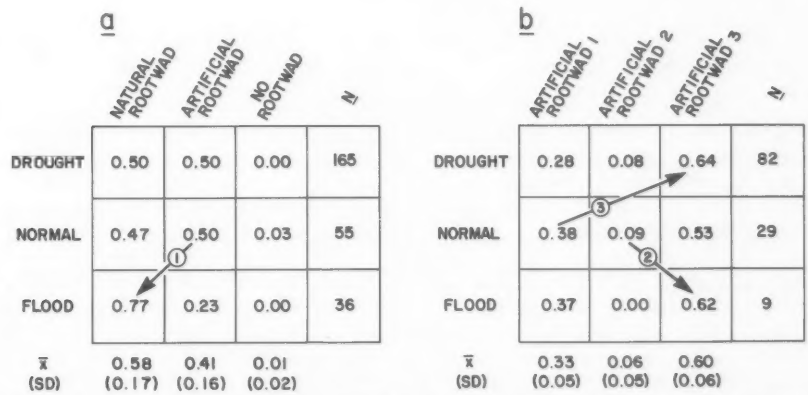

FIG. 5. Mean proportions of the total number of coho salmon fry observed downstream of three categories of rootwad abundance during drought (N = 6), normal (N = 3), and flood (N = 3) streamflows. Changes in distribution which occurred when streamflow changed are indicated by arrows numbered for reference from text. N = total number of fish counted for each streamflow level. Note that panel b is a breakdown of data in the second column of panel a.

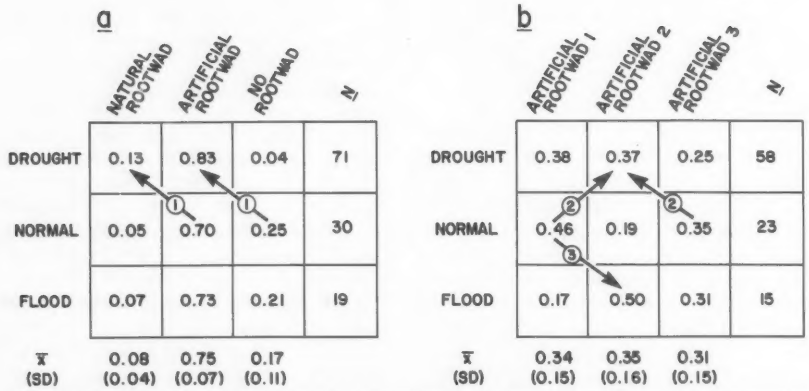

FIG. 6. Mean proportions of the total number of steelhead parr observed downstream of three categories of rootwad abundance during drought ($N=6$), normal ($N=3$), and flood ($N=3$) streamflows. Changes in distribution which occurred when streamflow changed are indicated by arrows numbered for reference from text. N = total number of fish counted for each streamflow level. Note that panel b is a breakdown of data in the second column of panel a.

increased from normal to flood flows, coho fry redistributed themselves so that their abundance decreased downstream of the artificial rootwads (t-test, $P=0.09$) and increased downstream of the natural rootwad closer toshore (t-test, $P=0.05$, arrow 1, Fig. 5). In addition to shifting to the position downstream of the natural rootwad, coho salmon fry redistributed themselves downstream of the artificial rootwads. When streamflow increased from normal to flood flows, the proportion of coho fry downstream from artificial rootwad 2 decreased (t-test, $P=0.21$) and the proportion downstream from artificial rootwad 3 increased (t-test, $P=0.40$, arrow 2, Fig. 5). In contrast, when streamflow decreased from normal to drought flows and conditions became shallower and slower instead of deeper and faster, coho salmon fry downstream of artificial rootwad 1 decreased (t-test, $P=0.21$) and increased downstream of artificial rootwad 3 (t-test, $P=0.36$, arrow 3, Fig. 5).

The steelhead parr used the rootwads in a different way than the coho salmon fry. Although they most frequently occupied positions downstream of the artificial rootwads, overall they chose the position without a rootwad more often than they chose the position of the natural rootwad (Fig. 6). When streamflow decreased from normal to drought flows, steelhead parr decreased their use of the position out in the open (t-test, $P=0.04$), and increased their use of positions downstream of both the natural and artificial rootwads (t-test, $P=0.17$, arrow 1, Fig. 6). That is, in response to conditions becoming shallower, slower, and brighter, the steelhead parr redistributed themselves to locations which were deeper, slower, and darker.

Relative Importance of the Habitat Variables

Stepwise regression analysis allowed ranking of the habitat variables in order of importance to the fish when they were selecting positions. For both the coho salmon fry and the steelhead parr, water velocity ranked first, water depth second, and light intensity third (Table 2). For coho salmon fry this ranking was unchanged regardless of whether the absolute light measurements or the standardized light measurements were used. For steelhead parr, however, conducting the regression analysis on the standardized, rather than on the absolute, light measurements caused the ranking of light to fall from most important to least important.

Discussion

In this study, 99% of the coho fry and 83% of the steelhead parr used positions downstream from natural or artificial rootwads in spring. Bustard and Narver (1975) found that locations under or downstream from upturned tree roots or logs were most frequently occupied by young salmon and trout in winter also. Moreover, Bustard and Narver (1975) found that while habitat choices of the two species were similar, juvenile steelhead most often chose positions associated with rocks and substrate, while juvenile coho chose positions more related to wood debris. Bugert (1985), using manipulation of wood debris in a natural stream much as in this study, also found that juvenile steelhead tended to select areas without wood and with higher water velocities more frequently than did juvenile coho salmon.

The reason juvenile coho salmon and steelhead trout so often select positions downstream from rootwads or other physical structures in streams has been speculated to be that these structures provide "cover" by shielding them from high water velocities (e.g. Bisson et al. 1987). By implication, positions without rootwads have water velocities which are too high to be usable. Less than 1% of the coho salmon fry and 17% of the steelhead parr occupied the no-rootwad position. Conditions at the no-rootwad position during the experiment, including water velocity, were the same as they were before the artificial rootwads were added. Consequently the frequency of occupation by coho salmon fry and steelhead parr of the no-rootwad position during the experiment indicates their likely abundance and distribution in the middle of the stream at the study location before the experiment began, plus their general use of mid-stream areas in Kloiya Creek without rootwads. The addition of artificial rootwads increased the use of mid-stream areas 3200% by coho salmon fry (Fig. 5) and 100% by steelhead parr (Fig. 6). This response suggests that the addition of wood to mid-stream areas could be a beneficial habitat enhancement technique in Pacific salmon streams. It remains uncertain, however, whether the addition of wood would actually increase the carrying capacity of the stream or simply result in a redistribution within the stream of the existing capacity.

Comparisons of the depth, velocity, and light intensity at the no-rootwad location (the conditions which would have existed

TABLE 2. Stepwise regression analysis of coho salmon fry and steelhead parr abundance with the water velocity, water depth, and standardized light intensity at positions they occupied during the experiment. Stepwise regression techniques used were forward, backward, and maximum r^2 improvement. All techniques gave the same variable ranking.

	Ranking[a]	Partial r^2[b]	Probability	Total r^2	Probability
Coho Salmon fry	1. Velocity	0.71	0.001	0.93	0.001
	2. Depth	0.15	0.37	0.22	0.54
	3. Standardized light intensity	0.14	0.12	0.18	0.20
Steelhead parr	1. Velocity	0.70	0.02	0.72	0.04
	2. Depth	0.00	0.91	0.50	0.18
	3. Standardized light intensity	0.19	0.02	0.20	0.04

[a]Standardizing light measurements changed variable ranking for steelhead parr from light first, velocity second, depth third, to velocity first, depth second, light third.

[b]Model used was $y = B_0 + B_1x_1 + B_2x_1^2 + E$. The partial r^2 reported in this table is that part contributed by the linear term of the model. The increase in the total r^2 from the partial r^2 is due to the additional contribution from the quadratic term of the model. In all cases, except depth for steelhead parr, the linear term contributed the majority of the variation explained by the model.

at the artificial rootwad locations before their placement) with the conditions downstream of the artificial rootwads shows that the artificial rootwads caused velocities and light intensities to decrease (Fig. 2). When the water velocities and light intensities decreased, coho salmon fry density increased from 0.21 fish per m^2 to 8.7 fish per m^2 and steelhead parr density increased from 1.7 fish to 7.5 fish per m^2 (Fig. 5a and 6a).

This increase in abundance is not direct evidence, but it appears likely that the increased use of mid-stream areas downstream of the artificial rootwads was because the artificial rootwads decreased the water velocity and light intensity at those locations. Evidence for this comes from the fishes' response to the changes in streamflow. For the coho salmon fry, when streamflow increased from normal to flood they moved to a location which was either deeper and slower (arrow 1, Fig. 5) or shallower and slower (arrow 2, Fig. 5). When streamflow decreased from normal to drought, they moved to a location which was shallower and slower (arrow 3, Fig. 5). The only consistent feature of the new locations selected was that the water velocity was slower. Lower water velocity was selected regardless of whether streamflow increased or decreased and irrespective of whether this required moving to a deeper or shallower location. Redistribution to new locations with slower water velocities has long been assumed to be the response of fish to increasing water velocities (Taylor 1988). The role of rootwads in relation to this behavior is commonly interpreted as the rootwad providing a shielding from adverse velocity (i.e. a shelter function) (Bisson et al. 1987). However, because the coho salmon fry also moved to slower locations in response to decreasing water velocity, it is possible the rootwads also provided a protection function in association with a fright response.

While the coho salmon fry appeared to select positions mostly on the basis of water velocity, the steelhead parr seemed to be much more influenced by light intensity. Although the steelhead parr most frequently occupied positions downstream of the artificial rootwads which suggests they may have been selecting positions shielded from the current (Fig. 6), they did not increase their use of positions downstream from rootwads when streamflow increased from normal to flood, something that would have been expected if they had been avoiding current. Their only significant redistribution occurred when streamflow decreased from normal to drought. When conditions became slower, shallower, and brighter, steelhead parr decreased their use of positions out in the open and increased their use of positions downstream from rootwads where light intensities were lower (arrow 1, Fig. 6).

Coho fry and steelhead parr appeared to select positions sometimes on the basis of water velocity and at other times on the basis of light intensity. If selection for water velocity is inferred to be related to shielding from adverse conditions, and selection for light intensity is inferred as related to avoidance of enemies, then rootwads at times provide a shelter function and at other times a protection function. If this explanation is correct, then the relative variation in fish numbers explained by the different variables in the regression analyses suggests that the coho salmon fry and steelhead parr selected positions associated with rootwads most often for shelter from current (roughtly 80% of the variation accounted for by water velocity, Table 2), and less often for protection by reduced visibility (20% of the variation accounted for by light intensity).

When considering water velocity and light intensity separately, it is not clear which variable is the most important to the fish in its selection of a station. It appears that at different streamflows different variables assume the greatest importance. The rankings calculated from the regression analyses for the variables in this study (Table 2) represented their relative importance overall, because they were calculated from all the fish responses to all levels of streamflow and wood abundance. Certain streamflows, however, appeared to alter or reverse the criterion on which the fish selected positions. The first example of this is movement of the steelhead parr in response to reduced streamflow. When conditions became slower and brighter, steelhead parr moved to a position of even lower velocity but darker (arrow 1, Fig. 6a). Even though water velocity ranked overall as the most important habitat variable in the regression analyses, in this case, the steelhead parr appeared to be selecting positions with preferred light intensity. If successful avoidance of detection by predators increases in reduced light intensities, then this evidence demonstrates that under certain conditions (in this case drought streamflows) protection, temporarily at least, may override considerations such as shelter from current.

A second case where the most important variable for position choice was not water velocity was the distribution of coho fry

downstream of the artificial rootwads. Coho fry occupied positions downstream from artificial rootwad 1 much more frequently than downstream from artificial rootwad 2 (3.3 fish per m^2 versus 0.6 fish per m^2 respectively, Fig. 5). This was despite artificial rootwad 1 having a much faster and therefore, presumably, less preferred velocity at normal and flood streamflows (Fig. 2). Consequently, coho fry did not appear to have selected positions downstream from artificial rootwad 1 for shelter from current. It was not possible to identify any physical habitat variable that could account for the observed distribution. The low utilization by coho fry of artificial rootwad 2 despite its more desirable water velocity may have been due to the presence of the larger steelhead parr at artificial rootwad 2. This location was preferred by the steelhead at drought and flood streamflows and one they moved to when streamflow either increased or decreased from normal (Fig. 6b, arrows 2 and 3). Social interaction is a factor in habitat utilization that must be taken into account when habitat preferences are being inferred from fish distribution.

These two examples show that positions downstream from rootwads are not selected exclusively for shelter from current, but that sometimes their function may be for protection. Since rootwads provide functions other than shelter from current, these examples also show that rootwads are important habitat structures not just during floods, but during droughts as well.

The results of this study show that rootwads can provide both shelter and protection simultaneously for fish with different motives. Although coho salmon fry and steelhead parr have preferred levels of water velocity and light intensity (Table 1), I suggest that the preferred levels may change depending on the activity the fish is engaged in. Movement by the steelhead parr to areas of lower velocity upon a reduction in streamflow (Fig. 6a) suggests that the steelhead's optimum level of water velocity may have shifted to a new, lower level when they shifted their activity for habitat selection from shelter to protection. If the steelhead parr altered their habitat preferences in response to a change in streamflow, then this has important significance for habitat protection and conservation. Many habitat assessment methods such as Physical Habitat Simulation (PHABSIM, Bovee 1982) and Habitat Evaluation Procedures (HEP, Raleigh 1978) assume that individual fish will select "optimum" levels of habitat variables and that these optimum levels are either constant or change only as the individual grows. Habitat models currently do not allow for shifts in the habitat preference by fish. If such shifts actually occur, then they may be the cause of the often inconsistent correlations between fish biomass and "cover" provided by wood found in some habitat models (Shirvell 1989).

It is my opinion that fish in this study were not selecting positions because of an affinity for rootwads – they were selecting positions because of an affinity for the conditions caused by the rootwads. Despite artificial rootwad 1 simulating the natural rootwad by causing similar depth, velocity, and light intensity at every streamflow, coho salmon fry still selected levels of these variables available downstream from artificial rootwad 3 preferentially over the levels of these variables available downstream from artificial rootwad 1. This demonstrates that they were selecting desirable levels of physical variables, not rootwads, and that when they could get more desirable levels of these variables they would move to them regardless of whether they were created by tree roots or warehouse pallets. It also demonstrates that they would move to desirable levels of these variables, regardless of their location, next to shore or away from shore.

Habitat assessment frequently includes judgement of the availability of "cover" provided by wood, including rootwads (Bisson et al. 1981). The complex way different levels of habitat variables can provide shelter and protection to fish has allowed carelessness to enter into these assessments. Cover objects (physical structures that cause the conditions which allow fish to be protected and sheltered) are often confused with cover habitat (the conditions themselves). As these experiments have shown, fish do not select cover objects, they select cover habitat: preferred levels of velocity, depth, light intensity, and other variables within the constraints of social interaction which allow them to be protected and sheltered. Rootwads, and rocks, and undercut banks are structural elements of fish habitat; cover is a function provided by these structural elements. Cover is a concept of how we think fish perceive and use parts of their habitat. Is wood in streams cover habitat? No. Wood in streams creates cover habitat.

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Increased Juvenile Salmonid Growth by Whole-River Fertilization

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Nutrient concentrations, periphyton standing crop and size of steelhead trout (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) fry increased after the fertilization of a nutrient-deficient stream with inorganic phosphorus (P) and nitrogen (N). Whole-river fertilization of the Keogh River, British Columbia, during 1983–86 to increase summer average nutrient concentrations from <1 μg $P \cdot L^{-1}$ and 25 μg $N \cdot L^{-1}$ to 10–15 μg $P \cdot L^{-1}$ and 30–100 μg $N \cdot L^{-1}$ resulted in five- to 10-fold increases in periphyton standing crops on artificial substrata and 1.4- to 2.0-fold increases in late-September salmonid fry weights. Diatoms and chlorophytes dominated the algal periphyton on artificial substrata at fertilized sites. Cyanophytes were unimportant despite low N:P ratios in some cases. Juvenile salmonids fed primarily on benthic insects. These results suggest that autochthonous primary production can be an important energy source in forested, middle-order streams, and indicate that the manipulation of autochthonous primary production can be a useful management tool to increase salmonid growth in nutrient-poor coastal streams.

Les teneurs en éléments nutritifs, la population exploitable du périphyton et la taille des truites arc-en-ciel (*Oncorhynchus mykiss*) et des alevins de saumon coho (*O. kisutch*) ont augmenté après fertilisation en phosphore (P) et azote (N) inorganique d'un cours d'eau pauvre en éléments nutritifs. La fertilisation de toute la rivière Keogh en Colombie-Britannique entre 1983 et 1986, en vue d'accroître les teneurs estivales moyennes en éléments nutritifs de <1 μg $P \cdot L^{-1}$ et 25 μg $N \cdot L^{-1}$ à 10–15 μg $P \cdot L^{-1}$ et 30–100 μg $N \cdot L^{-1}$ a entraîné des augmentations de 5- à 10-fois de la population exploitable du périphyton sur les substrats artificiels et de 1,4- à 2,0-fois dans les poids des alevins de salmonidés à la fin de septembre. Aux sites fertilisés, les diatomés et les chlorophycées étaient les plus abondants du périphyton d'algues sur les substrats artificiels. Les cyanophycées étaient plus nombreuses malgré les rapports N :P faibles dans certains cas. Les salmonidés juvéniles se nourrissaient essentiellement d'insectes benthiques. Ces résultats semblent montrer que la production primaire autochtone peut constituer une source énergétique importante des cours d'eau moyens, en milieu boisé, et indiquent que la manipulation de la production primaire autochtone peut être un outil de gestion utile en vue d'augmenter la croissance des salmonidés dans des cours d'eau côtiers pauvres en éléments nutritifs.

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Positive correlations between indices of nutrient concentrations and fish production have long been noted in streams (McFadden and Cooper 1962; Egglishaw 1968) and lakes (Northcote and Larkin 1956; Ryder et al. 1974). Recently, the fertilization of oligotrophic, salmon-nursery lakes has produced large increases in adult returns (LeBrasseur et al. 1978), largely through the effects of increased food abundance on smolt size and consequent marine survival (Hyatt and Stockner 1985). Huntsman (1948) suggested that inorganic fertilization could increase fish production in streams. The ultimate effect of fertilization is to increase the abundance of fish food-organisms which, in low- and middle-order streams, are mainly the immature stages of aquatic insects. Increases in the standing stocks of stream zoobenthos after organic enrichment have repeatedly been demonstrated in experimental streams and troughs (Warren et al. 1964; Mundie et al. 1973; Williams et al. 1977; Mundie et al. 1983), but there are few experimental studies of the effects of inorganic enrichment.

This neglect may stem from the belief that energy flow through forested stream ecosystems is largely driven by the import of allochthonous organic material, especially autumnal leaf litter (Hynes 1975; Boling et al. 1975; Anderson and Sedell 1979), despite evidence of the importance of autochthonous (algae) energy pathways in many streams (Minshall 1978). The relative importance of allochthonous and autochthonous energy sources will vary with nutrient and light levels. Inorganic nutrient additions can increase periphyton production in nutrient-deficient streams (Stockner and Shortreed 1978; Peterson et al. 1983; Perrin et al. 1987). Peterson et al. (1985) demonstrated a change from heterotrophy to autotrophy by the addition of phosphorus to a tundra stream. Fertilization of the tundra stream with phosphorus also increased the size of larval

blackflies (Peterson et al. 1985) and *Orthocladius* larvae (Hershey et al. 1988). Because some benthic algae are a high-quality food for aquatic insects (Cummins and Klug 1979), and the numbers of aquatic herbivores may be regulated by the amount of algae available (Gregory 1983), the abundance of stream insects should be increased by inorganic fertilization.

Food abundance, acting through territory size, may be an important factor influencing the production of stream-dwelling salmonids (Chapman 1966; Slaney and Northcote 1974; Dill et al. 1981). Positive correlations between measures of food abundance in streams and both salmonid biomass (Murphy et al. 1981; Bowlby and Roff 1986) and growth rates (Warren et al. 1964; Wilzbach et al. 1986) support this view. Increased food availability may increase both the abundance (Slaney and Northcote 1974; Mason 1976; Wilzbach 1985) and size (Mason 1976; Wilzbach et al. 1986) of stream-rearing salmonids. Increased adult returns with increasing smolt size are reported for several stream-rearing anadromous salmonids, such as coho salmon (*Oncorhynchus kisutch*) (Hager and Noble 1976) and steelhead trout (*O. mykiss*, formerly *Salmo gairdneri*) (Ward and Slaney 1988). Thus, the fertilization of nutrient-deficient streams to increase food availability may be a promising technique for increasing coho salmon and steelhead trout production.

We examined the effects of whole-river enrichment with inorganic phosphorus and nitrogen fertilizers on the size of juvenile coho salmon and steelhead trout that reared in a small, coastal, British Columbia river. We also examined associated changes in periphyton accrual, zoobenthos standing crop and the diet of steelhead and coho salmon fry to provide information on the processes leading to increased fish size. A more detailed account of the effects of organic and inorganic enrichment on periphyton accrual in experimental sections of the stream is given in Perrin et al. (1987) while a preliminary account of the effects of whole-river fertilization on juvenile steelhead is given in Slaney et al. (1986).

Materials and Methods

Study Site

The enrichment experiments took place in the Keogh River (127°25′W by 50°35′N), a third-order coastal stream at the northeastern end of Vancouver Island, British Columbia (Fig. 1). The Keogh River is 32 km long and drains a watershed of about 130 km^2. Although the stream originates from a lake, stream flow is variable, especially during the winter rainy period. Mean annual discharge is 5.3 $m^3 \cdot s^{-1}$, while the maximum estimated flow is 254 $m^3 \cdot s^{-1}$. Mean summer flows are about 1.6 $m^3 \cdot s^{-1}$ near the mouth, and minimum summer flow is about 0.1 $m^3 \cdot s^{-1}$. Wetted width at mean summer flow varies from 5 m in the headwaters to 15 m at the river mouth. Riffles, pools, and runs make up 41, 22, and 37% of the river length, respectively (Ward and Slaney 1979). The substratum is mainly gravel and cobble.

About 35% of the watershed has been logged during the last 30 yr. The headwater areas upstream of km 29 (Fig. 1) have been logged to the streambank, while other areas have streamside buffer strips or are unlogged. The riparian vegetation in logged areas is red alder (*Alnus rubra*), salal (*Gaultheria shallon*), willow (*Salix* spp.), sedges and grasses. Western hemlock (*Tsuga heterophylla*), red cedar (*Thuja plicata*), and sitka spruce (*Picea sitchensis*) dominate the mature forests.

FIG. 1. Keogh River drainage, showing the locations of sampling sites and fertilizer dispensers in 1984.

Canopy closure is about 30% in the headwaters and 60 to 70% elsewhere. During the fertilization study, logging in the vicinity of the river occurred only in late summer 1985, at isolated sites near km 23 and km 3.7.

Ambient nutrient concentrations are low. Soluble reactive phosphorus (SRP) and total dissolved phosphorus concentrations are <1 and about 5 $\mu g \cdot L^{-1}$, respectively during spring and summer months. Dissolved inorganic nitrogen (DIN; nitrate + nitrite + ammonia) concentrations are also low. Nitrate plus nitrite is usually less than 15 $\mu g\ N \cdot L^{-1}$ while ammonia ($NH_3 + NH_4^+$) is less than 5 $\mu g\ N \cdot L^{-1}$. Inorganic P and N concentrations increase slightly with declining flows through the summer, to maxima of 3 $\mu g\ P \cdot L^{-1}$ and 60 $\mu g\ N \cdot L^{-1}$ (Perrin et al. 1987). Mean pH is 6.9, total alkalinity is 7.0 $mg \cdot L^{-1}$ as $CaCO_3$ and total dissolved solids are about 30 $mg \cdot L^{-1}$.

The Keogh River has estimated annual runs of 2000 to 33 000 (odd year) and 25 000 to 60 000 (even year) pink salmon (*O. gorbuscha*); in recent years, odd and even year runs have been similar (Johnston et al. 1986). Spawning pink salmon occur upstream to km 30.5, our control site. Returns of other anadromous salmonids are about 1000 to 3000 coho salmon, 200 to 4600 steelhead trout (wild plus hatchery fish, 1976 to 1988) and 150 to 250 chum salmon (*O. keta*). Anadromous Dolly Varden char (*Salvelinus malma*) and resident cutthroat trout (*O. clarki*, formerly *Salmo clarki*) also occur. An average of 71 000 coho salmon smolts (Irvine and Ward 1989) and 5500 steelhead smolts (Ward and Slaney 1988) are produced annually.

Fertilization Treatments

Periphyton growth in the Keogh River is phosphorus-limited at ambient nutrient concentrations (Perrin et al. 1987). Target nutrient concentrations during fertilization were chosen to saturate P-limited periphyton growth rates while simultaneously satisfying known tissue N:P ratios. In all cases (except possibly 1983, see below), summer average phosphorus concentrations in fertilized sections (Fig. 3) were greater than levels known to saturate the biomass accrual of lotic periphytic diatom communities (Bothwell 1989). Thus, although several concentrations of fertilizer were applied throughout the experiment, we regard these as a single treatment, "inorganic fertilization," applied in 5 yr. Differences in fertilizer applications were primarily in loadings of inorganic nitrogen.

In 1981, we fertilized 300-m sections of the upper Keogh River. During 1983 to 1986, the lowermost 29 km of river were continuously fertilized with N and P throughout the summer growing season while the upper 3 km remained as an untreated control reach. In 1976 to 1980, 1982, and 1987 to 1988 the river was not fertilized.

The effects of organic and inorganic enrichments were studied between April and September, 1981, in four similar 300-m sections of the upper Keogh River between km 30.5 and km 29 (Fig. 1). A detailed description of the experimental procedure is given in Perrin et al. (1987). Briefly, from mid-May to mid-September the four stream sections were subject to: no treatment (CONTROL); a monthly addition of 280 $g \cdot m^{-2}$ rolled barley (total P = 0.40%, total N = 1.88%, total C = 39.24%) (GRAIN); continuous additions of inorganic fertilizers (N-P-K formulations of 34-0-0 and 11-55-0) to produce ambient concentrations of 15 $\mu g\ P \cdot L^{-1}$ and 200 $\mu g\ N \cdot L^{-1}$ (LOWPN); and inorganic fertilizer additions to produce concentrations of 20 $\mu g\ P \cdot L^{-1}$ and 400 $\mu g\ N \cdot L^{-1}$ (HIPN). Because of low natural recruitment of steelhead trout, all sections were stocked with steelhead trout fry (Keogh River stock, mean weight 0.40 g) on 8 June at about 0.3 $fish \cdot m^{-2}$. Adjacent treatments were separated by 300-m buffer zones, which were considered adequate as the median dispersal distance of stocked steelhead fry in higher gradient coastal streams is usually less than 150 m (Hume and Parkinson 1987).

From 1983 to 1986, inorganic P and N were added to produce target P concentrations of 10 to 15 $\mu g \cdot L^{-1}$ in the 29-km treatment reach. N loadings varied, as we wanted to reduce the application of this expensive material to the minimum required to generate a strong response by the periphyton. In 1983 a solid prill, slow-release fertilizer (Osmocote®, Sierra Chemical Ltd., Milpitas, Calif.) was broadcast by helicopter over the 29-km treatment reach on three occasions between 24 March and 25 July to produce target concentrations of 10 $\mu g\ P \cdot L^{-1}$ and 100 $\mu g\ N \cdot L^{-1}$. In 1984, the Keogh River downstream of km 29 was continuously fertilized with inorganic P and N from 4 May to 28 September. Two fertilization techniques were used. Osmocote® prill was broadcast by hand in the upper 3 km of the fertilized section on three occasions, to produce ambient P and N concentrations of 15 $\mu g \cdot L^{-1}$. The remainder of the river was continuously fertilized with a mix of 11-55-0 and 34-0-0 agricultural fertilizers using mechanical dispensers (Perrin et al. 1987) located at 3-km intervals, to produce concentrations of 15 $\mu g \cdot L^{-1}$ P and N in the upper 3 km or 15 $\mu g\ P \cdot L^{-1}$ and 150 $\mu g\ N \cdot L^{-1}$ in the lower 23 km. In 1985, the 29-km treatment reach was continuously fertilized from 15 April to 27 September, using mechanical dispensers located at approximately 5-km intervals, to produce concentrations of 10 $\mu g\ P \cdot L^{-1}$ and 100 $\mu g\ N \cdot L^{-1}$. In 1986, the 29-km enrichment section was continuously fertilized with 11-55-0 fertilizer from 28 April to 10 September, using mechanical dispensers at approximately 5-km intervals, to produce a concentration of 10 $\mu g\ P \cdot L^{-1}$. The lowermost 26 km was additionally fertilized with 34-0-0 fertilizer to give a nitrogen concentration of 50 $\mu g\ N \cdot L^{-1}$.

Fertilization Effects on Fry Size

We assessed the effects of inorganic fertilization on the weights of age 0+ coho salmon and steelhead trout fry with a "Before-After Control-Impact" experimental design (Stewart-Oaten et al. 1986) that compared the differences between paired measurements in control and fertilized reaches during treated and nontreated time periods. Analyses were done on log-transformed data to improve normality and homogeneity of variances. The additivity and independence assumptions of the analysis were tested using the single degree of freedom test for nonadditivity (Tukey 1949) and von Neumann's ratio test (von Neumann 1941; Hart 1942).

We determined mid-summer (late July to early August) fry weights in the treatment and control reaches from 1976 to 1988 and "end-of-growing-season" (late September to early October) weights from 1983 to 1988 by sampling at a series of locations within each reach. At each sampling location, we censused fish populations in several adjacent habitats, each about 10 to 15 m long, sealed with stop nets. Fish numbers were estimated by the method of Seber and Le Cren (1967), based on three or four electrofishing circuits at each site using a Smith-Root (Vancouver, Washington) type VIIIA electroshocker. We measured wet weights to the nearest 0.1 g for all parr and random subsamples of fry, and fork lengths to the nearest millimeter for all fish. The weights of unweighed fry were estimated from site-specific, functional regressions (Ricker 1973) of log(weight) against log(length). Fry were separated from parr by scale aging and length-frequency analysis. Steelhead parr were aged from plastic impressions of scale samples.

Mean fry weights at the several sampling locations within each experimental unit (control or treatment reach) were averaged to give a reach mean, as recommended by Hurlbert (1984) and Stewart-Oaten et al. (1986), because locations were subsamples drawn from a single experimental unit. Although location differences undoubtedly exist and the set of locations sampled varied somewhat among years, this would tend to increase the variability in the data, yielding a more conservative test of fertilization effects.

Response Mechanisms

To provide information on the mechanisms leading to a fish response, we collected ancillary data on nutrient concentrations, periphyton accrual, benthos standing crop, and fish diet at various times throughout the study. Because we lack a time-series of data in control and fertilized reaches during nontreatment and treatment periods, the ancillary data cannot unequivocally separate fertilization effects from intrinsic location differences or from temporal trends that differentially affected control and treatment reaches. Nevertheless, the data are useful in elucidating the probable responses at other trophic levels.

Water samples for the determination of nutrient concentrations were collected and processed as described by Perrin et al. (1987). Sample sites in the treatment reach were located in rif-

fles several kilometers downstream of fertilizer dispensers. Nutrient concentrations were measured throughout the summer in control and fertilization reaches in four pretreatment years (1975 to 1978) and in all fertilization years. Detection limits were 3 $\mu g \cdot L^{-1}$ for SRP and 20 $\mu g\ N \cdot L^{-1}$ for nitrate from 1975 to 1978, and 1 $\mu g \cdot L^{-1}$ for SRP and 5 $\mu g\ N \cdot L^{-1}$ for nitrate or ammonia from 1981 onward.

Periphyton accrual on styrofoam substrata was measured at the control site and several treatment sites in several times series during each of the fertilization years, following the procedures of Perrin et al. (1987). Periphyton standing crop was measured as chlorophyll *a*. Taxonomic analyses used the Utermöhl technique at 500 × magnification on Lugol-preserved samples (Perrin et al. 1987). Periphyton accrual on plexiglass substrata (Stockner and Shortreed 1976) was measured at the control and one treatment site during 1978.

During the 1981 experiment, benthos samples were collected at approximately monthly intervals at the CONTROL, GRAIN, and HIPN sites, using a Hess sampler of 0.05 m^2 with a 200 μm mesh net. The substratum was sampled to 10 cm depth during base flow conditions, in water depths of 15 to 45 cm. A set of five samples was taken at each of two riffles within each treatment section on each date, and preserved in 10% formalin. Samples were hand-picked at 10 × magnification, with a sorting efficiency >90%. Invertebrates were identified to the family level; insect taxonomy follows Merritt and Cummins (1978). Wet weights were measured to the nearest milligram on an electronic balance after blotting the organisms on filter paper to remove excess preservative. Shells were removed from bivalves prior to weighing.

Fry diet was monitored during 1984 and 1985 to assess feeding on benthic insects capable of responding to fertilization. In 1984, steelhead and coho fry were collected for diet analysis in mid-June and late September by electroshocking during the day. The fish were preserved in 10% formalin, the stomachs later excised, and the contents removed and identified from 10 fry of each species at each site. In 1985, fish diet was monitored in the control and fertilized reaches from monthly samples collected by electroshocking at dusk.

Results

Fish Growth

Inorganic fertilization resulted in striking increases in the weights of steelhead and coho salmon fry in fertilized areas (Fig. 2) during all years of treatment, except for coho fry in 1983 when nutrient concentrations were considerably below target values and the periphyton response was considerably reduced.

During the eight nontreatment years before and after fertilization, mid-summer steelhead fry (logarithmic) mean weights were generally slightly higher in the control reach than in the fertilization treatment reach (Fig. 2a). During the 5 yr of fertilization treatment, however, the mid-summer logarithmic mean weights of steelhead fry were considerably greater in the fertilized reach than in the control reach (Fig. 2a). The mean of the differences between the treatment and control reaches during the fertilization years was significantly greater than the mean of the differences during the nontreatment years ($t = 7.22$, $df = 11$, $p < 0.001$, one-tailed). That is, steelhead fry weights were significantly increased by the fertilization treatment. The additivity and independence assumptions of the above analysis were met. During each of the nontreatment and fertilization time periods, the difference in mean weights between control and fertilized reaches was constant (Tukey's single degree of freedom test for nonadditivity, $p = 0.27$ and $p = 0.13$, respectively). Similarly, the temporal replicates were independent within each period (von Neumann ratio test, $k = 2.7$, $n = 8$, $p \gg 0.06$ and $k = 1.15$, $n = 5$, $p > 0.07$).

Fall steelhead fry weights showed a similar response (Fig. 2b), the mean of the differences between treatment and control reach averages being significantly elevated during fertilization ($t = 3.05$, $df = 4$, $p = 0.019$, one-tailed). The additivity and independence assumptions were met in the fertilization replicates (Tukey test for nonadditivity, $p = 0.13$; von Neumann ratio test, $k = 1.12$, $n = 4$, $p > 0.06$), but the assumptions could not be tested for the nontreatment data ($n = 2$).

Coho fry mid-summer weights also increased in the treatment reach during fertilization (Fig. 2c). The mean of the differences between treatment and control reaches in fertilized years was significantly greater than the mean of the differences during nonfertilized years ($t = 3.65$, $df = 11$, $p = 0.002$, one-tailed), and the additivity and independence assumptions were met (Tukey test, $p = 0.68$ and $p = 0.97$; von Neumann test, $k = 3.2$, $n = 8$, $p \gg 0.06$ and $k = 3.1$, $n = 5$, $p \gg 0.06$ for nontreatment and fertilization years, respectively). However, the mean of the differences between the fertilization reach and control reach coho fry weights at the end of the growing season did not differ significantly between the fertilization and nonfertilization periods ($t = 1.06$, $df = 4$, $p = 0.17$, one-tailed), although fall coho fry weights were generally greater in the treatment reach during fertilization (Fig. 2d). The additivity and independence assumptions were again satisfied (Tukey test, $p = 0.92$; von Neumann test, $k = 1.44$, $n = 4$, $p > 0.07$ for fertilization replicates). The apparent lack of response in the fall coho weights may result from the low statistical power of the test ($1 - \beta \approx 0.23$). Thus, it is quite possible that fall coho fry weights did increase in the treatment reach during fertilized years (Fig. 2d) but that we were unable to detect the increase statistically. If the ineffective 1983 treatment is not considered, the likelihood that fall coho fry weights were elevated during fertilization is considerably increased ($t = 2.04$, $df = 3$, $p = 0.067$, one-tailed) despite the low power of the test ($1 - \beta \approx 0.47$).

The average effect of inorganic fertilization was to increase steelhead trout fry geometric mean weights about 95% and coho salmon fry geometric mean weights about 40% by the end of the growing season (Table 1). Most of the weight gain was achieved by mid-summer (Table 1).

Water Chemistry and Periphyton Accrual

Prior to whole-river fertilization, DIN and SRP concentrations were low and similar in all reaches of the Keogh River (Fig. 3). During whole-river fertilization, DIN and SRP values remained low in the control area but were considerably elevated in the fertilized reach. The apparent decrease in nutrient concentrations at the control site from 1981 onward (Fig. 3) resulted from a decrease in detection limits. N:P atomic ratios in unfertilized stream sections ranged between 20:1 and 60:1 during fertilization, which suggests that algal production would potentially be phosphorus-limited.

Summer average nutrient concentrations in fertilized reaches generally were at or above target values except in 1983, when Osmocote™ was used as the nutrient source. Although Osmo-

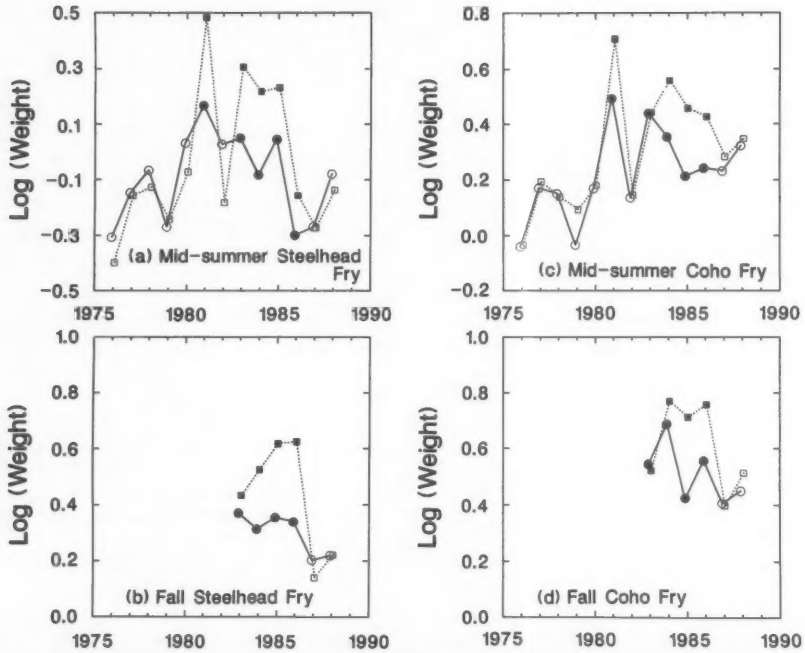

FIG. 2. Logarithmic mean weights (g) of salmonid fry in control (○) and treatment (□) reaches of the Keogh River during nontreatment (open symbols) and fertilization (filled symbols) periods: (a) steelhead fry in mid-summer 1976–88, (b) steelhead fry at the end of September 1983–88, (c) coho salmon fry in mid-summer 1976–88, and (d) coho salmon fry at the end of September 1983–88.

TABLE 1. Geometric mean weights (g) of steelhead trout and coho salmon fry at mid-summer and late September in control and fertilization areas of the Keogh River during treatment and nontreatment periods.

	Unfertilized years	Fertilized years	Difference
	Mid-summer		
Steelhead			
Fertilized sites	0.63	1.65	+1.02
Control sites	0.73	0.95	+0.22
Difference	−0.10	+0.70	+0.80
Coho			
Fertilized sites	1.47	3.30	+1.83
Control sites	1.38	2.22	+0.84
Difference	+0.09	+1.08	+0.99
	Late September		
Steelhead			
Fertilized sites	1.52	3.55	+2.03
Control sites	1.63	2.21	+0.58
Difference	−0.11	+1.34	+1.45
Coho			
Fertilized sites	2.86	4.90	+2.04
Control sites	2.68	3.57	+0.89
Difference	+0.18	+1.33	+1.15

cote[TM] was chosen to provide a constant rate of release over the inter-treatment periods, the nutrients were released immediately after application as a pulse of short duration. Consequently, average nutrient concentrations were considerably lower (Fig. 3) than the target concentrations of 10 μg $P \cdot L^{-1}$ and 100 μg $N \cdot L^{-1}$. Phosphorus concentrations during 1983 were frequently lower than those required to saturate algal standing crop (Bothwell 1989); ambient P was less than the 1 μg $P \cdot L^{-1}$ detection limit in 46% of the samples from the fertilized reach.

Whole-river fertilization during 1984 to 1986 affected algal periphyton similarly to inorganic fertilizer additions to 300-m stream sections in 1981 (Perrin et al. 1987). Prior to fertilization, periphyton standing crops were low and similar at control and treatment sites. Chlorophyll *a* standing crops were less than 5 mg chl $a \cdot m^{-2}$ at all sites (Fig. 4). During fertilization in 1984 to 1986, standing crops were as much as an order of magnitude greater at fertilized sites than at the control (Fig. 4). Standing crops reached maximum values of 100 to 200 mg chl $a \cdot m^{-2}$ at fertilized sites in late spring and early summer, but only 10 to 20 mg chl $a \cdot m^{-2}$ at the control site. Maximum standing crops were lower in late summer, but the large differences between the control and fertilized sites were generally maintained. Periphyton standing crop was somewhat lower in 1983, when target concentrations of nutrients were not achieved. Maximum chl *a* values at treated sites in 1983 (50 to 60 mg chl $a \cdot m^{-2}$) were about five times those at the control, and differences between the control and treatment sites were not consistently maintained (Fig. 4).

The attached algal community at the control site was dominated by diatoms throughout the entire study (Table 2). Diatoms were also the dominant group at the fertilized sites, but chlorophytes were a much more important component of the periphyton at fertilized sites than at the control, especially in late summer (Table 2). Cyanophytes were always a minor component of the periphyton. *Tabellaria, Synedra, Fragilaria,* and

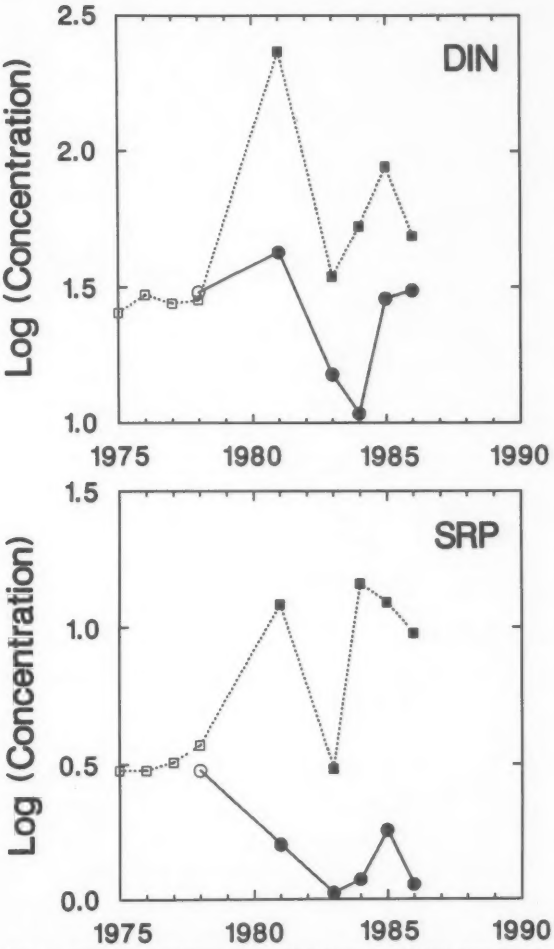

FIG. 3. Summer logarithmic mean SRP (lower) and DIN (upper) concentrations ($\mu g \cdot L^{-1}$) in control (○) and fertilized (□) reaches of the Keogh River during nontreatment (open symbols) and fertilization (filled symbols) periods.

Achnanthes were abundant diatom genera at all sites. Other common genera such as *Cocconeis, Cymbella, Navicula,* and *Diatoma* were abundant only at the fertilized sites. *Cosmarium* and *Closterium* were common chlorophytes at all sites. The increases in chlorophytes seen in late summer largely comprised filamentous forms such as *Ulothrix, Oedogonium,* and *Spirogyra* in 1984 but *Ankistrodesmus, Scendesmus,* and *Spirogyra* in 1985. *Oscillatoria* and *Anabaena* were the only bluegreens found.

Benthic Invertebrates

There were few large differences in the taxonomic composition of benthos samples among the control, organic enrichment and high inorganic fertilization sites in the 1981 experiment. The benthic community was typically dominated by baetid, leptophlebid, and hepatgeniid mayflies; chironomid and simuliid flies; chloroperlid stoneflies; hydropsychid caddisflies; and elmid beetles.

Mean standing crops of benthic invertebrates were similar at all sites during the first 2 mo of enrichment in 1981 (Fig. 5). Thereafter, the standing crops at the grain and inorganic treatment sites continued to increase while that at the control site declined slightly. By the end of the enrichment period in September, standing crops of benthos at the grain and inorganic tretament sites were three to five times that of the control (Tukey hsd test, $p<0.05$). Higher density of benthos in the organic enrichment section relative to the control resulted from higher densities of chironomid and simuliid larvae, while that at the inorganic fertilization site resulted from higher abundances of chironomid and hydropsychid caddisfly larvae. Stoneflies were also more abundant at both enriched sites. Over the enrichment period, standing crops of benthos at the enrichment sections averaged about twice that in the control section.

Fry Diet

The stomach contents of steelhead fry in 1984 and 1985 consisted primarily of mayfly nymphs and dipteran larvae (Fig. 6), which were also the most abundant taxa in the benthos when examined in 1981. Mayflies were more heavily utilized at the control site, while dipteran flies comprised more of the diet at the fertilized sites. Terrestrial insects were usually a minor component of the diet. Caddisfly larvae and stonefly nymphs increased in importance at all sites in late September. Baetidae were the most abundant mayfly in steelhead fry stomachs, but Heptageniidae, Ephemerellidae, and Leptophlebidae were occasionally abundant. Chironomids were the most common dipteran fly larvae.

The diet of coho salmon fry was more diverse, but mayflies and dipterans, especially baetid and heptageniid nymphs and chironomid larvae, again were the most common prey items (Fig. 6). Terrestrial insects also formed a significant fraction of the diet. Caddisflies and stoneflies were occasionally important.

Discussion

Fertilization of the nutrient-deficient Keogh River with inorganic P and N resulted in significant increases in the mean weights of juvenile salmonids. Substantial increases in periphyton standing crop also occurred during fertilization. These changes indicate that the manipulation of inorganic nutrient concentrations to increase autochthonous primary production can be a useful technique to increase fish growth in nutrient-deficient streams.

Periphyton Response

The results of the Keogh River fertilization experiment are important in resolving concerns about extrapolating trough experiment results to the ecosystem level (Peterson et al. 1983), and in confirming the magnitude of algal responses to enrichment in a nutrient-deficient stream: large increases in periphyton standing crop can be produced stream-wide in P-limited streams.

The responses of periphytic algae in the Keogh River to whole-river enrichment were qualitatively and quantitatively similar to those seen in smaller-scale enrichment experiments in other nutrient-deficient streams. The five- to 10-fold increases in periphyton standing crop in fertilized sections of the Keogh River were similar to those observed in trough experiments in Carnation Creek, B.C., when P was added (Stockner

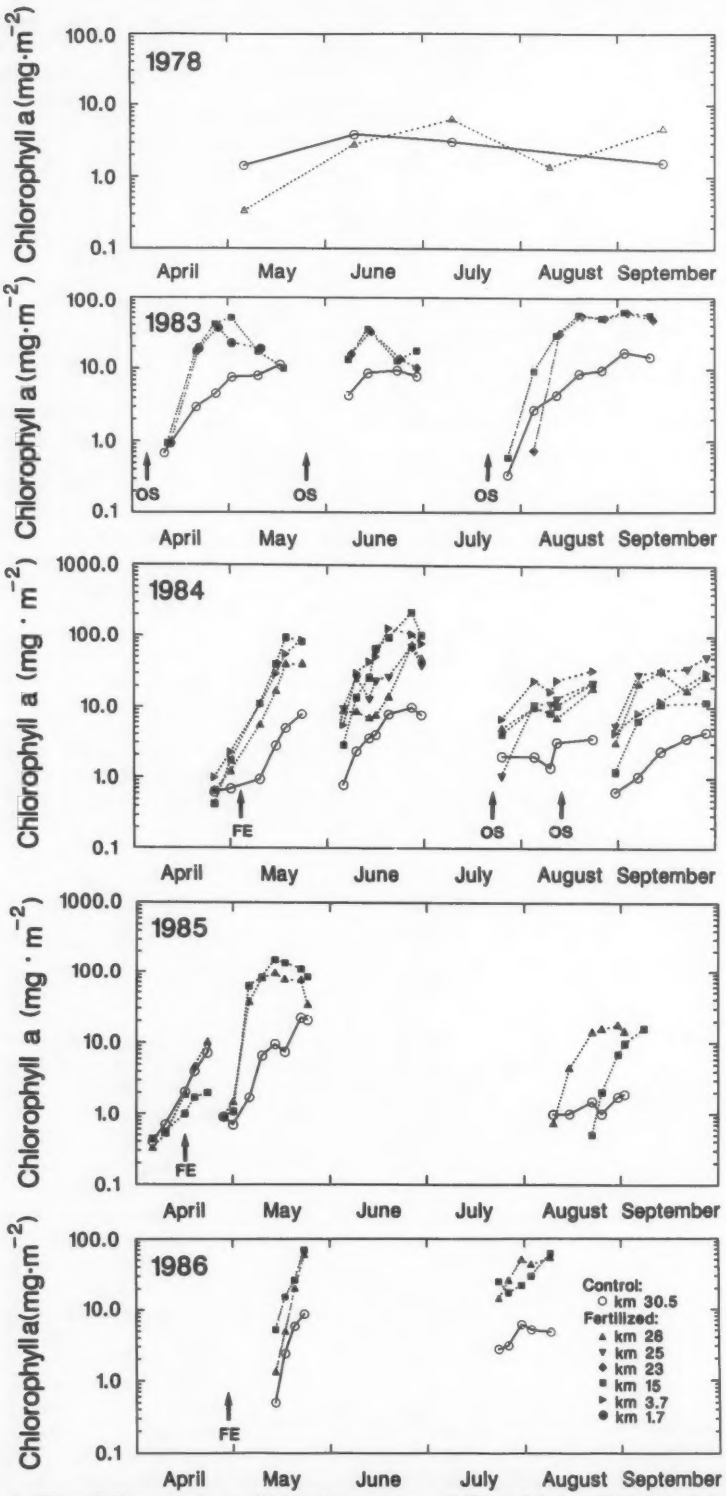

FIG. 4. Chlorophyll *a* accrual on artificial substrata prior to (1978) and during (1983–86) whole-river

TABLE 2. Average percent volume by major taxonomic group of periphytic algae on styrofoam substrata at control and fertilized sites in the Keogh River during whole-river fertilization, 1984 to 1986. + indicates presence in trace amounts.

	May		June		August		September	
	Control	Fert	Control	Fert	Control	Fert	Control	Fert
1984								
Bacillariophyceae	99	95	99	83	92	72	95	68
Chlorophyta	<1	3	<1	14	8	28	4	25
Cyanophyta	+	<1		3		+		2
Chrysophyta								5
1985								
Bacillariophyceae	99	93					72	46
Chlorophyta	1	6					28	54
Cyanophyta		1					+	+
Chrysophyta								
1986								
Bacillariophyceae					95	95		
Chlorophyta					5	5		
Cyanophyta						+		
Chrysophyta								

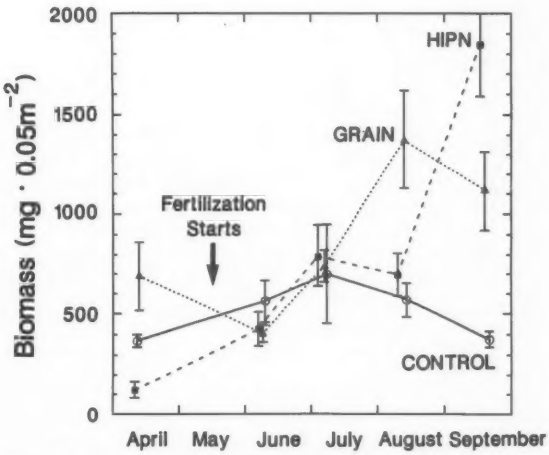

FIG. 5. Changes in benthic biomass (mean ± SE, $n = 10$) at 300 m control, organic enrichment (GRAIN), and high inorganic enrichment (HIPN) stream sections during the 1981 enrichment experiment.

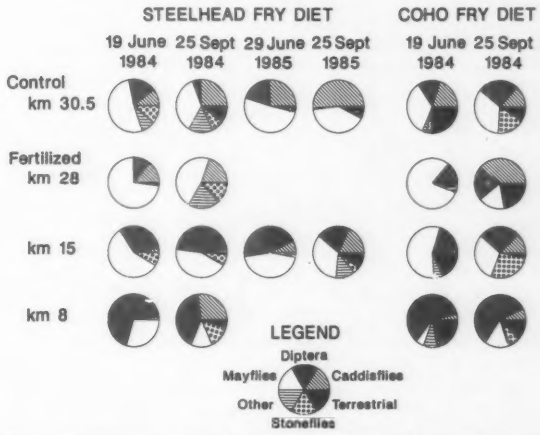

FIG. 6. Percent composition by numbers of taxa in the stomach contents of steelhead trout and coho salmon fry at control and fertilized sites in the Keogh River during the 1984 and 1985 whole-river fertilization treatments.

and Shortreed 1978). Phosphorus enrichment of experimental tubes in a tundra stream also produced similar increases in chl *a* levels (Peterson et al. 1983). Ambient concentrations of SRP were about 10 $\mu g \cdot L^{-1}$ in all three studies. However, much smaller changes in chl *a* were reported during P enrichment of 120–150 m sections of a deciduous woodland stream (Elwood et al. 1981), possibly as a result of simultaneous N limitation.

Nitrogen limitation was not a major concern in augmenting autochthonous production in the Keogh River at the levels of P enrichment used. Although seasonal average N:P atomic ratios varied from 2:1 to 16:1 among different fertilized areas, greatly increased chl *a* levels occurred in all treatments. Periphyton accrual on styrofoam substrata was similar over a wide range of DIN concentrations (Fig. 4). In contrast, Stockner and Shortreed (1978) and Peterson et al. (1983) obtained larger increases in chl *a* levels when both N and P were added than when either nutrient was added alone. Also, despite low average N:P atomic ratios in some cases, blue-green algae did not become an important part of the periphyton, as predicted by Stockner and Shortreed (1978) for N:P ratios below 5:1. Presumably, natural sources of N were a sufficient supply; DIN in unfertilized areas averaged about 25 $\mu g\ N \cdot L^{-1}$. Fertilization

FIG. 4. (continued)
fertilization of the Keogh River. The initial datum in each series was taken 4 to 7 d after the commencement of the accrual measurements. FE indicates the start of fertilization and OS indicates the addition of Osmocote®.

TABLE 3. Densities and geometric mean wet weights of salmonid fry in control and fertilized stream sections after 2.5 mo of treatment, July 1981. 95% confidence limits and sample sizes in brackets. $n = 4$ for the density estimates.

	Weight (g)	Density (fish·m^{-2})
Steelhead fry		
Control	1.5 (1.2–1.8, $n = 33$)	0.15 (0.11–0.21)
Organic	1.9 (1.7–2.2, $n = 41$)	0.22 (0.18–0.27)
Low inorganic	3.2 (2.9–3.5, $n = 47$)	0.27 (0.0–0.55)
High inorganic	2.9 (2.6–3.3, $n = 30$)	0.11 (0.04–0.17)
Coho salmon fry		
Control	3.1 (2.7–3.5, $n = 40$)	0.28 (0.0–0.70)
Organic	3.4 (3.1–3.9, $n = 56$)	0.32 (0.02–0.63)
Low inorganic	5.3 (4.9–5.7, $n = 57$)	0.36 (0.15–0.57)
High inorganic	5.0 (4.7–5.3, $n = 90$)	0.33 (0.03–0.63)
Dolly Varden fry		
Control	0.7 (0.2–2.2, $n = 3$)	0.014 (0.0–0.03)
Organic	1.5 (1.3–1.7, $n = 20$)	0.082 (0.0–0.19)
Low inorganic	2.1 (1.5–2.9, $n = 14$)	0.079 (0.02–0.14)
High inorganic	2.3 (1.9–2.7, $n = 13$)	0.049 (0.0–0.12)
Weight multiple comparisons (Tukey hsd test)		
Steelhead fry	Control<GRAIN<LOPN = HIPN	
Coho salmon fry	Control = GRAIN<LOPN = HIPN	
Dolly Varden fry	Control<GRAIN = LOPN = HIPN	

increased the relative abundance of chlorophytes in the Keogh River, but the periphyton community remained dominated by a few genera of diatoms, such as *Achnanthes, Fragilaria, Synedra,* and *Tabellaria,* which also became common in Carnation Creek when nutrients were added (Stockner and Shortreed 1978).

Our results (Fig. 4) suggested that ambient SRP concentrations of 10 to 15 μg P·L^{-1} are needed to saturate periphyton standing crop. We measured lower periphyton standing crops in 1983, when ambient P averaged 3.0 μg P·L^{-1} in the fertilized section, than in 1984 to 1986 when ambient P was 10 to 15 μg P·L^{-1}. The sustainable biomass of diatom-dominated stream periphyton communities is known to saturate at relatively high phosphorus levels (Bothwell 1989), although growth at the cellular level saturates at much lower P concentrations (0.3 to 0.6 μg P·L^{-1}, Bothwell 1988).

Fish Response

We attribute the larger fish sizes at fertilized sites to increased food availability. Thus, we hypothesize that elevated nutrient concentrations during the fertilization treatment increased periphyton standing crop, which in turn led to increased production of the benthic insects that the fry consumed.

Our data were consistent with this view, although only the changes in fish size can be ascribed unequivocally to the fertilization treatment. During fertilization, both nutrient concentrations and periphyton standing crop were higher in the fertilized reach than in the control reach. Both steelhead and coho salmon fry fed primarily on benthic insects. In the 1981 enrichment experiment, salmonid fry were largest in the treated sections (Table 3), in which benthos standing crops and the abundances of those benthic insects consumed by salmonid fry were highest. Coho fry, which utilized terrestial prey to a greater extent than did steelhead fry, showed a smaller response to inorganic fertilization. In 1983, when both nutrient concentrations and periphyton standing crop in the fertilized area were lower than in other treatment years, the differences in fish sizes between the control and fertilized reaches were also reduced.

The data suggest that autochthonous primary production can be an important energy source in forested, middle-order coastal streams. The immediate changes in fish size observed during fertilization indicated that the benthic insect community, on which the juvenile salmonids fed, may respond quickly to alterations of food abundance.

It is likely that increased periphyton standing crops would lead to increased production of benthic insects. The increased standing crop of periphytic diatoms found in fertilized areas of the Keogh River would increase food availability or quality for many aquatic insects (Anderson and Cummins 1979; Bird and Kaushik 1984). Decreased mortality, increased growth, and aggregation of aquatic insects are expected in organically enriched substrates (Mundie et al. 1983). Increased food quality or availability can also alter the fecundity and generation length of aquatic insects (Anderson and Cummins 1979; Sweeney et al. 1986). Accelerated development and increased body size were seen in the dipterans *Orthocladius* and *Prosimulium* following fertilization of a tundra stream (Peterson et al. 1985; Hershey et al. 1988). Our 1981 data indicated higher standing crops of benthic invertebrates in the enriched stream sections than in the control at the end of the summer, but it was unclear whether the differences represented treatment or location effects.

The immediate increases in salmonid fry sizes during fertilization also suggested that fry growth was strongly food-limited. Several experimental studies have directly demonstrated the importance of food availability in determining the growth rates of stream-dwelling salmonids. Mason (1976) found that the production of juvenile coho was food-limited during the summer, and that both size and density were increased several-fold by experimental feeding. Wilzbach et al. (1986) found that short-term growth rates of cutthroat trout were correlated with invertebrate drift densities. Murphy et al. (1981) also found that trout biomass in riffles was positively correlated with benthos density, and that the growth rates of fry varied directly with insect drift rates in several small Oregon streams. Warren et al. (1964) observed seven-fold higher production of cutthroat trout in experimental streams whose benthic invertebrate populations had increased four-fold after enrichment with sucrose.

The growth of stream-rearing salmonids may also be influenced by population density (e.g. Fraser 1969; Randall 1982) acting through territory size. Territory size is itself strongly influenced by food availability (Slaney and Northcote 1974). Dill et al. (1981) observed that territory size in a natural population of coho fry was negatively correlated with the density of benthos on the territories. The increased fish sizes observed at our fertilized sites were not likely to result from differences in fry density rather than increased food abundance. Some of the inter-site differences seen during 1976 to 1988 may have resulted from differences in fry abundance, but it is unlikely that the among-year pattern of variation in fry density at fertilized and control sites exactly duplicated our treatments. In the 1981 experiments, juvenile salmonid densities were very similar at all sites (Table 3) but mean weights differed substantially.

Other data indirectly confirm our conclusion that the fertilization treatment was responsible for the observed increases in salmonid fry sizes. First, the size-at-age of emigrating steelhead smolts increased and the mean age decreased for brood years

subject to fertilization (Slaney et al. 1986). Second, the proportion of steelhead parr older than 1+ years decreased in the fertilized area compared with both the control and the prefertilization data (Slaney et al. 1986). These results strongly suggest that whole river fertilization has led to increased fish growth that has been maintained over several years of treatment.

The increases in steelhead trout and coho salmon fry sizes that resulted from the fertilization treatment could have important effects on smolt output from the river, since overwinter survival increases with fry size in coho salmon (Holtby and Hartman 1982) and in stocked steelhead (Hume and Parkinson 1988). In turn, increased smolt numbers and size are likely to increase adult returns, since smolt-to-adult survival increases with smolt size in steelhead (Ward and Slaney 1988) and in coho salmon (Hager and Noble 1976).

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Aspects of the Energy Metabolism of Lake Sturgeon, *Acipenser fulvescens*, with Special Emphasis on Lipid and Ketone Body Metabolism

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Key enzymes in several metabolic pathways in five tissues were measured in a primitive osteichthyan, the lake sturgeon (*Acipenser fulvescens*). Levels of nonesterified fatty acids (NEFAs) were measured in the plasma as an indicator of fatty acid mobilization and differential utilization of individual NEFAs as substrates for lipid oxidation. The metabolism of lake sturgeon differs from that of most teleosts studied; it has detectable levels of beta-hydroxybutyrate dehydrogenase in all tissues, possibly a primitive metabolic feature of vertebrates, subsequently lost in the teleosts. Based on HOAD and CPT activities lipid oxidation in extrahepatic tissues of sturgeon is intermediate between elasmobranch and teleost models. Sturgeon plasma NEFA concentrations are clearly higher than those detected in any elasmobranch, indicating that the acipenserid chondrosteans may be among the first jawed fish to mobilize and transport NEFAs. Oleic acid (18:1,*n*9) which amounts to 45% of total NEFA content of the plasma may be a preferred substrate of carnitine dependent oxidation. High levels of long chain fatty acid in the plasma may represent a high turnover of eicosanoid precursors. The low levels of LDH in the anoxia tolerant sturgeon may indicate that alternative anaerobic end products, perhaps ethanol, are used to survive under anoxic conditions.

Les principales enzymes de plusieurs voies métaboliques dans cinq tissus ont été mesurées dans un ostéichthyen primitif, l'esturgeon de lac (*Acipenser fulvescens*). Les concentrations d'acide gras nonesterifiés (NEFA) ont été mesurées dans le plasma à titre d'indicateur de la mobilisation de l'acide gras et de l'utilisation différentielle de chaque NEFA comme substrats pour l'oxydation des lipides. Le métabolisme de l'esturgeon de lac diffère de celui de la plupart des téléostéens étudiés; ce poisson affiche en effet des concentrations détectables de beta-hydroxybutyratedéhydrogénase dans tous ses tissus, ce qui était probablement une caractéristique métabolique primitive des vertébrés, disparue par la suite chez les téléostéens. D'après les activités de HOAD et de CPT, l'oxydation des lipides dans les tissus extrahétapiques de l'esturgeon se situe à un niveau intermédiaire entre celle des modèles élasmobranches et téléostéens. Les concentrations plasmatiques de NEFA de 'esturgeon sont de toute évidence plus élevées que celles relevées chez tous les élasmobranches, ce qui révèle que les chondrostéens acipensérídés pourraient avoir été parmi les premiers poissons à mâchoires à mobiliser et à transporter les NEFA. L'acide oléique (18:1, *n*9), qui constitue jusqu'à 45 % de la teneur totale en NEFA du plasma, pourrait être un des substrats préférés de l'oxydation liée à la carnitine. Les fortes concentrations plasmatiques d'acides gras à longue chaîne pourraient témoigner d'un fort taux de renouvellement des précurseurs éicosanoïdes. Les faibles concentrations de LDH chez l'esturgeon tolérant l'anoxie pourraient indiquer que d'autres produits finals anaérobies, peut-être l'éthanol, sont habitués de survivre en conditions anaxiques.

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Sturgeon are of biological interest for several reasons. Firstly, sturgeon occupy an important evolutionary position intermediate between the elasmobranchs and the teleosts. Secondly, sturgeon are highly prized as food fish. Thirdly, and most importantly, their worldwide numbers are declining.

Sturgeons are the sole living representatives of an ancient group of fishes known as the acipenserid chondrosteans. They are thought to have descended from the freshwater palaeoniscids of the upper Devonian (360 million years ago) giving rise to a new group of Osteichthyans, the ray-finned fish or Actinopterygians (Carroll 1988). It is from this group that the presently successful teleosts are derived. The chondrosteans were most abundant during the Carboniferous with only two orders, the Polypteriformes and the Acipenseriformes, surviving today.

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The latter order contains the families Acipenseridae and Polyodontidae. Both families have retained several primitive morphological characters, clearly distinguishing them from the modern teleosts. Among the primitive characteristics retained by sturgeons are the large body shields or plates, a large cellular swimbladder, a cartilaginous skeleton, a shark-like heterocercal caudal fin, and a notochord persistent in the adult form (Scott and Crossman 1973). These anatomical differences have been used to place the sturgeon in an intermediate evolutionary position between elasmobranchs and teleosts.

In many areas of the world sturgeon are prized for the commercial value of their flesh and roe. Over-exploitation, habitat destruction, and pollution have endangered many species of sturgeon (Doroshov 1985; Brousseau 1987). One species in particular is endangered; lake sturgeon *Acipenser fulvescens* populations in Ontario have declined rapidly since the turn of the century (Harkness and Dymond 1961; Brousseau 1987; Houston 1987). Slow sexual maturation, high natural mortality of juveniles and sensitivity to fishing gear, allows little chance for rapid recovery of sturgeon stocks after depletion resulting from overharvesting (Semakula and Larkin 1968; Houston 1987). To restore natural populations, several species have been artificially cultured (Doroshov et al. 1983; Ceskleba et al. 1985; Smith 1985; Smith et al. 1985).

Much of the research relating to the culture of sturgeon has focused on: (1) reproductive biology, (2) nutritional requirements for growth of young sturgeon, and (3) environmental effects on growth of young (see Conte et al. 1987 for review). Aside from a few studies of sturgeon osmoregulatory (Potts and Rudy 1972; McEnroe and Cech 1985) and respiratory physiology (Burggren 1978; Burggren and Randall 1978; Cech et al. 1984; Ruer et al. 1987) there have been no investigations of the metabolic organization of sturgeon. There is evidence to suspect that sturgeon may have a metabolic organization different from that of teleost fish. Recent studies (Zammit and Newsholme 1979; Ballantyne et al. 1987; Singer and Ballantyne 1989) indicate the existence of marked differences between the metabolic organization of the phylogenetically ancient elasmobranchs and teleosts. These include the following three differences: (1) extrahepatic lipid oxidation in elasmobranchs is reduced compared with teleosts (Zammit and Newsholme 1979; Driedzic and Hart 1984; Ballantyne and Moon 1986; Singer and Ballantyne 1989). (2) There is a lack of detectable levels of beta-hydroxybutyrate dehydrogenase (β-OHBDH) in teleosts, compared with substantial levels of this enzyme in freshwater (Singer and Ballantyne 1989) and marine elasmobranchs (Zammit and Newsholme 1979). This enzyme is required for oxidation of the ketone body beta-hydroxybutyrate. The lack of β-OHBDH in teleosts is accompanied by lower blood concentrations of all ketone bodies and the complete lack of blood beta-hydroxybutyrate compared with elevated levels in elasmobranchs (Zammit and Newsholme 1979). (3) There are 10-fold lower concentrations of plasma non-esterified fatty acids (NEFAs) in elasmobranchs compared with teleosts (Zammit and Newsholme 1979).

The metabolic differences between the more "primitive" elasmobranchs and the "advanced" teleosts suggest that certain osteichthyans which occupy intermediate phylogenetic positions may have a metabolic organization of lipid and ketone body oxidation that is intermediate between that of the teleost and the elasmobranch. We, therefore, examined the capacity of selected tissues of a primitive osteichthyan, the lake sturgeon, for lipid and ketone body oxidation. The activities of various enzymes of intermediary metabolism, particularly those involved in the metabolism of lipids and ketone bodies, were measured. Levels of NEFAs were measured in the plasma as an indicator of the extent of fatty acid turnover (mobilization and oxidation). In addition levels of certain long chain fatty acids, specifically arachidonic acid (AA, 20:4*n*6), eicosapentoaenoic acid (EPA, 20:5*n*3) and docosahexaenoic acid (DHA, 22:6*n*3), were also measured in the plasma to examine their role as potential eicosanoid precursors. Such investigations not only contribute to the understanding of the evolution of fish metabolism but also provide the basis for improved sturgeon culture techniques required by enhancement programs and the aquaculture industry for this threatened species.

Materials and Methods

Experimental Animals

Lake sturgeon were obtained from a commercial fisherman from Lake Huron and held at the Marine Biology Laboratory, University of Guelph, in freshwater at 10° (±1.0°)C for 2 wk prior to use. Animals were fed ad libitum on a commercial salmonid diet daily (Diet 84G, Martin Feed Mills Ltd, Elmira, Ontario) and were observed to feed. Fish were immature and weighed 2.0 to 6.0 kg.

Tissue Preparation

Animals were killed by a blow to the head and tissues were removed and placed in 5 volumes of ice cold 50 mM imidazole buffer, pH 7.4, at 10°C. All preparative procedures were carried out at 4.0°C. Tissues were homogenized using a Polytron PT10 unit (Kinematica Gmbh., Luzurn, Switzerland) for three bursts lasting 10 s each time. Homogenates were centrifuged at 18 200 g for 20 min at 4°C and an aliquot of the resulting supernatant was used directly in enzyme assays. For assays of carnitine palmitoyltransferase (CPT), carnitine octanoyltransferase (COT), carnitine acetyltransferase (CAT), and citrate synthase (CS), Triton X-100 was added to the homogenate to make a 0.2% solution, which was then sonicated using a Megason PA-300 unit (Ultrasonic Instruments International, Farmingdale, NY, USA) for three bursts of 10 s each at the highest setting prior to use. This method gave the highest levels of CPT in tissues of *Raja erinacea* (J. S. Ballantyne and J. A. Berges, unpubl. data) and lake charr, *Salvelinus namaycush* (T. D. Singer and J. S. Ballantyne, unpubl. data).

Enzyme Assays

Maximal enzyme activities were determined using a Varian DMS 100 UV-Visible spectrophotometer (Varian Canada Inc., Georgetown, Ont., Canada) equipped with thermostated cell changer maintained at 10° (±0.1°) C with a Haake D8 circulating water bath (Haake Buchler Instruments Inc., Saddlebrook, NJ, USA). Reaction rates of most enzymes were determined by change in absorbance of NADH or NADPH at 340 nm (millimolar extinction coefficient ϵ_{340}, 6.22). Citrate synthase (CS) and the carnitine acyl transferases were monitored at 412 nm using 5,5′ dithiobis 2-nitrobenzoic acid (DTNB) (millimolar extinction coefficient ϵ_{412}, 13.6). Acetoacetyl-CoA thiolase (ACoAT) was measured at 313 nm using the method of Williamson et al. (1971) (millimolar extinction coefficient ϵ_{313}, 20.5). Succinyl coenzyme-A ketotransferase (SKT) was monitored at 310 nm (millimolar extinction coef-

ficient ϵ_{310}, 11.9) using the method of Moon and Mommsen (1987). Enzyme activities are expressed as units per gram wet tissue weight where one unit equals 1 μmol substrate converted to product per minute.

Conditions of the enzyme assays were optimized with respect to substrate concentration and are as follows:

Enzymes of lipid catabolism:

Carnitine palmitoyltransferase (CPT; E.C. 2.3.1.21): 50 mM imidazole, pH 8.0, at 20°C, 5 mM L-carnitine, 0.2 mM (DTNB), 0.1 mM palmitoyl CoA (omitted for control).

Carnitine octanoyltransferase (COT; E.C. 2.3.1.21): 50 mM imidazole, pH 8.0, at 20°C, 5 mM L-carnitine, 0.2 mM (DTNB), 0.1 mM octanoyl CoA (omitted for control).

Carnitine acetyltransferase (CAT; E.C. 2.3.1.7): 50 mM imidazole, pH 8.0, at 20°C, 5 mM L-carnitine, 0.2 mM (DTNB), 0.1 mM acetyl CoA (omitted for control).

3-hydroxyacyl CoA dehydrogenase (HOAD; E.C. 1.1.1.35): 50 mM imidazole, pH 8.0, at 20°C, 0.1 mM NADH, 0.1 mM acetoacetyl CoA (omitted for control).

Enzyme of lipid synthesis:

Malic enzyme (ME; E.C. 1.1.1.40): 50 mM imidazole, pH 7.4, at 10°C, 1.0 mM $MgCl_2$, 0.4 mM NADP, 1.0 mM malate (omitted for control).

Enzymes of ketone body metabolism:

β-hydroxybutyrate dehydrogenase (β-OHBDH; E.C. 1.1.1.30): 50 mM imidazole, pH 8.0, at 20°C, 0.1 mM NADH, 2 mM acetoacetate (omitted for control).

Succinyl coenzyme-A ketotransferase (SKT; E.C. 2.8.3.2):50 mM imidazole, pH 8.0, at 20°C, 5 mM $MgCl_2$, 0.1 mM acetoacetyl CoA, 1 mM succinate (omitted for control).

Acetoacetyl-CoA thiolase (ACoAT; E.C. 2.3.1.9): 50 mM imidazole, pH 8.0, at 20°C, 5 mM $MgCl_2$, 0.14 mM acetoacetyl CoA, 0.24 mM CoA (omitted for control).

Enzyme of oxidative metabolism:

Citrate synthase (CS; E.C. 4.1.3.7): 50 mM imidazole, pH 8.0, at 20°C, 0.1 mM DTNB, 0.3 mM acetyl CoA, 0.5 mM oxaloacetate (omitted for control).

Enzymes of glycolysis:

Hexokinase (HK; E.C. 2.7.1.1): 50 mM imidazole, pH 7.4, at 20°C, 1 mM glucose, 5 mM $MgCl_2$, 0.16 mM NADP, 2 units glucose 6-phosphate dehydrogenase (G6PDH), 1 mM ATP (omitted for control).

Phosphofructokinase (PFK; E.C. 2.7.1.11): 50 mM imidazole, pH 7.4, at 20°C, 10 mM $MgCl_2$, 50 mM KCl, 0.15 mM NADH, 2 mM ATP, 1 unit α-glycerophosphate dehydrogenase, 1 unit triosephosphate isomerase, 1 unit aldolase, 5 mM D-fructose 6-phosphate (omitted for control).

Lactate dehydrogenase (LDH; E.C. 1.1.1.27): 50 mM imidazole, pH 7.4, at 20°C, 0.2 mM NADH, 1 mM pyruvate (omitted for control).

Enzyme of gluconeogenesis:

Fructose 1,6-biphosphatase (FBPase; E.C. 3.1.3.11): 50 mM imidazole, pH 7.4, at 20°C, 15 mM $MgCl_2$, 0.2 mM NADP, 2 units G6PDH, 10 units phosphoglucose isomerase, 0.1 mM fructose 1,6 diphosphate (omitted for control).

Enzymes of amino acid metabolism:

Glutamate dehydrogenase (GDH; E.C. 1.4.1.3): 50 mM imidazole, pH 7.4, at 20°C, 250 mM ammonium-acetate, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM NADH, 1 mM ADP, 14 mM α-ketoglutarate (omitted for control).

Alanine aminotransferase (AlaAT; E.C. 2.6.1.2): 50 mM imidazole, pH 7.4, at 20°C, 200 mM L-alanine, 0.2 mM NADH, 0.025 mM pyridoxal phosphate, 1 unit LDH, 10.5 mM α-ketoglutarate (omitted for control).

Aspartate aminotransferase (AspAT; E.C. 2.6.1.1): 50 mM imidazole, pH 7.4, at 20°C, 7 mM α-ketoglutarate, 0.2 mM NADH, 0.025 mM pyridoxal phosphate, 3 units malate dehydrogenase, 39.1 mM L-aspartate (omitted for control).

Determination of Plasma Nonesterified Fatty Acids (NEFAs)

Blood was drawn into heparinized syringes by caudal puncture before sacrificing the fish. The plasma was obtained by centrifugation of whole blood at 4°C and stored at −20.0°C until use. Nonesterified fatty acids (NEFAs) in plasma samples were assayed essentially by the method of Lepage and Roy (1988) with minor modifications (V. G. Mahadevappa, unpubl. data). One of the advantages of this assay is that it allows a quantitative determination of individual NEFAs in small aliquots of plasma. The interference by fatty acids that are esterified to plasma phospholipids and cholesterol esters is very minimal and the quantitative recoveries of NEFAs are highly reproducible between samples (85–95%) (Lepage and Roy 1988; V. G. Mahadevappa, unpubl. data). Selective methylation of NEFAs was achieved in the presence of methanol-acetyl chloride mixture (50:1). A known amount of internal standard (15 μg), heptadecanoic acid (17:0), was also methylated along with plasma NEFAs. Plasma samples (150 μL) containing a known amount of internal standard and methanol-acetyl chloride (5 mL) were placed in glass tubes equipped with tightly fitting teflon-lined caps and the tubes were maintained at 24–29°C with constant stirring for 45 min in a Reacti-Therm heating/stirring dry block (Pierce Chemical Co., Rockford, IL, USA). The above temperature and duration of heating need to be strictly maintained to prevent any esterified fatty acids being transmethylated during this reaction (V. G. Mahadevappa, unpubl. data). At the end of the methylation, 6% potassium carbonate solution (3mL) was slowly added to the mixture followed by the addition of hexane (150 μL). The tubes were shaken and centrifuged for 10 min at 2000 g. The hexane upper phase containing methyl esters of NEFAs was removed carefully with a pasteur pipette and placed in a small glass vial. Hexane was then evaporated under oxygen-free nitrogen gas and methyl esters were redissolved in 60 μL carbon disulfide (CS_2). One-half μL CS_2 containing methyl esters was injected into the gas chromatograph (Hewlett-Packard, HP5890A) fitted with a flame ionization detector (FID). Fatty acid methyl esters were analyzed on a DB-225 megabore fused silica column (Chromatographic specialities Inc., Brockville, Ont., Canada) under isothermal conditions (200°C) using helium as a carrier gas (Mahadevappa and Powell 1989). The identification of unknown fatty acid methyl esters was accomplished by comparing their retention times with known standards and the absolute amounts of these fatty acids were determined with the aid of internal standard, heptadecanoic acid (17:0).

Chemicals

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and were of the highest purity available.

Results

The key enzymes of several metabolic pathways were assayed in brain, red muscle, heart, kidney, and liver (Table 1) to provide a qualitative view of the metabolic organization of the lake sturgeon.

Lipid Metabolism

The activity of HOAD, an enzyme involved in beta-oxidation of fatty acids, was found in all tissues examined although the activity varied between tissues. For example, red muscle, heart, and kidney enzyme activity was greater than that found in liver and brain which had the lowest activity of this enzyme. However, CPT, an enzyme which catalyzes the rate-limiting step in carnitine dependent oxidation of long chain fatty acids (McGarry and Foster 1980), was detected only in the heart, red muscle, and brain whereas COT and CAT activities were present in all tissues examined. The liver and heart had the highest activity of malic enzyme, an enzyme associated with fatty acid synthesis, whereas the brain had the lowest activity of malic enzyme.

Table 2 summarizes the absolute amounts of individual nonesterified fatty acids (NEFAs) detected in the plasma of lake sturgeon. Chain lengths shorter than C:14 were not resolved under our conditions, and thus are not reported. The total NEFAs (C:14 and longer) in plasma of the lake sturgeon amounted to 2.493 μmol/mL. Monoenes were the major fatty acids detected and contributed 60% of the total. Oleic acid (18:1) accounted for 45% of the total NEFAs found in the plasma of the lake sturgeon. Palmitic acid (16:0) accounted for 17% of the NEFAs. The ratio of *n*3/*n*6 fatty acids in the plasma was 1.8.

Ketone Body Metabolism

Ketone body oxidation, as measured by activity of beta-hydroxybutyrate dehydrogenase which oxidizes beta-hydroxybutyrate transported in the blood to acetoacetate in the mitochondria, was low but measurable in both hepatic and extrahepatic tissues. Correspondingly, SKT, an enzyme which catlyzes the transfer of coenzyme A from succinyl coenzyme A to acetoacetate to form acetoacetyl coenzyme A, was detectable in all tissues with heart and red muscle having highest activity and liver and brain the lowest levels. ACoAT, an enzyme which catalyzes the cleavage of acetoacetyl coenzyme A to form acetyl coenzyme A, was found in all tissues but was highest in the liver and kidney.

Oxidative Metabolism

Based on levels of CS, a component enzyme of the Krebs cycle indicative of oxidatitive metabolism, the most aerobic tissue was found to be the heart and the lowest the liver.

Glycolysis

Glycolytic capacity was low based on HK, PFK, and LDH activities. Hexokinase phosphorylates glucose while phosphofructokinase phosphorylates fructose-6-phosphate in the formation of acetyl coenzyme A from glucose, thus aerobic metabolism of carbohydrates. Hexokinase and phosphofructokinase were variable but generally low in all tissues. Lactate

TABLE 1. Enzyme activities in selected tissues of the Lake Sturgeon *Acipenser fulvescens*.[a] ND, not detectable. For all other abbreviations, see text.

Enzyme	Activity ($\mu mol \cdot min^{-1} \cdot g^{-1}$)[b]				
	Brain	Red muscle	Heart	Kidney	Liver
Lipid catabolism					
HOAD	0.052 ± 0.007	0.339 ± 0.060	0.189 ± 0.069	0.260 ± 0.037	0.182 ± 0.022
CPT	0.002 ± 0.002	0.039 ± 0.018	0.054 ± 0.033	ND	ND
COT	0.039 ± 0.027	0.070 ± 0.015	0.033 ± 0.017	0.078 ± 0.026	0.066 ± 0.027
CAT	0.023 ± 0.006	0.056 ± 0.018	0.010 ± 0.009	0.084 ± 0.022	0.019 ± 0.008
Lipid synthesis					
Malic Enzyme	0.041 ± 0.005	0.225 ± 0.047	0.380 ± 0.046	0.132 ± 0.029	0.436 ± 0.077
Ketone body metabolism					
β-OHBDH	0.022 ± 0.008	0.029 ± 0.016	0.026 ± 0.015	0.013 ± 0.005	0.028 ± 0.012
SKT	0.014 ± 0.003	0.401 ± 0.112	0.431 ± 0.130	0.107 ± 0.029	0.024 ± 0.011
ACoAT	0.232 ± 0.054	0.888 ± 0.194	0.613 ± 0.134	1.113 ± 0.126	1.739 ± 0.223
Oxidative metabolism					
CS	3.269 ± 1.196	3.148 ± 0.707	7.497 ± 1.676	2.896 ± 0.393	0.502 ± 0.174
Glycolysis					
HK	0.662 ± 0.051	0.008 ± 0.002	0.754 ± 0.062	0.794 ± 0.064	0.184 ± 0.026
PFK	0.081 ± 0.019	0.488 ± 0.185	0.023 ± 0.016	0.100 ± 0.016	0.009 ± 0.006
LDH	60.33 ± 5.660	39.96 ± 8.670	38.20 ± 2.449	38.55 ± 4.468	16.68 ± 6.304
Gluconeogenesis					
FBPase	0.068 ± 0.048	0.094 ± 0.058	0.056 ± 0.041	0.131 ± 0.052	0.434 ± 0.070
Amino acid metabolism					
GDH	0.573 ± 0.136	1.985 ± 0.331	1.018 ± 0.143	2.187 ± 0.200	0.802 ± 0.191
AlaAt	2.297 ± 0.296	4.085 ± 0.303	0.789 ± 0.148	11.05 ± 1.193	8.427 ± 1.507
AspAT	6.597 ± 0.415	37.04 ± 5.201	26.63 ± 3.534	16.81 ± 0.955	16.67 ± 0.918

[a]Conditions were as described in Materials and Methods.
[b]Activities presented as mean ± SE; $n = 6$.

TABLE 2. Absolute amounts of individual nonesterified fatty acids in plasma of lake sturgeon *Acipenser fulvescens*. Values presented as mean ± SE; $n = 5$.

Fatty Acid	Concentration ($\mu mol \cdot mL^{-1}$)	Percentage (% by moles)
14:0	0.022 ± 0.004	0.92 ± 0.17
16:0	0.444 ± 0.058	17.72 ± 1.55
16:1	0.359 ± 0.038	14.35 ± 1.03
18:0	0.138 ± 0.009	5.65 ± 0.49
18:1(*n*9)	1.119 ± 0.072	45.08 ± 1.49
18:2(*n*6)	0.041 ± 0.003	1.68 ± 0.19
18:3(*n*3)	0.028 ± 0.005	1.10 ± 0.16
18:4(*n*3)	0.015 ± 0.003	0.58 ± 0.10
20:1	0.032 ± 0.003	1.30 ± 0.17
20:2(*n*6)	0.011 ± 0.003	0.48 ± 0.13
20:4(*n*6)	0.081 ± 0.005	3.30 ± 0.20
20:4(*n*3)	0.008 ± 0.003	0.32 ± 0.08
20:5(*n*3)	0.085 ± 0.014	3.34 ± 0.30
22:3(*n*3)	0.011 ± 0.003	0.39 ± 0.11
22:5(*n*3)	0.022 ± 0.002	0.88 ± 0.03
22:6(*n*3)	0.075 ± 0.014	2.91 ± 0.36
Total	2.493 ± 0.174	100
Total saturates	0.605 ± 0.069	24.29 ± 2.03
Total monoenes	1.511 ± 0.103	60.73 ± 2.07
Total polyenoic	0.377 ± 0.039	14.97 ± 0.60
(*n*3) Polyenoic	0.244 ± 0.039	9.51 ± 0.89
(*n*6) Polyenoic	0.133 ± 0.004	5.46 ± 0.37
(*n*3) / (*n*6)	1.83 ± 0.29	1.83 ± 0.29
(Monoenes/Polyenoic)	4.10 ± 0.24	4.10 ± 0.24

TABLE 3. Ratio of LDH/CS enzyme activity[a] in red muscle and heart of fishes.

Species	Assay temp(°C)	Red muscle	Heart
Cyclostomes			
Myxine glutinosa[b]	15	—	16.5
Elasmobranchs			
Raja erinacea[c]	15	—	13.6
Raja erinacea[d]	10	3.7	16.5
Squalus acanthias[c]	15	—	11.7
Potamotrygon magdalenae[e]	25		2.7
Chondrosteans			
Acipenser fulvescens (present study)	10	12.7	5.1
Teleosts			
Cyprinus carpio[f]	25	41.7	—
Cyprinus carpio[c]	15	—	41.3
Esox niger[g]	15	17.9[A] 34.7[B]	10.9[A] 17.1[B]
Salvelinus fontinalis[h]	15	—	13.7
Salmo salar[h]	15	—	5.8
Makaira nigricans[i]	25	33.6	49.8

[a]Activities ($\mu mol \cdot min^{-1} \cdot g^{-1}$).
[b]Values are from Hansen and Sidell (1983).
[c]Values are from Sidell et al. (1987).
[d]Values are from Moon and Mommsen (1987).
[e]Values are from Singer and Ballantyne (1989).
[f]Values are from Moyes et al. (1989).
[g]Values are Kleckner and Sidell (1985); acclimation temperature = [A]5°C, [B]25°C.
[h]Values are from Ewart and Dreidzic (1987).
[i]Values are from Suarez et al. (1986).

dehydrogenase, an enzyme involved in anaerobic metabolism, was highest in the brain and lowest in the liver. The ratio of LDH/CS activity, an indicator of anaerobic capacity, was lower in the heart compared with other species of fish except a freshwater elasmobranch, and intermediate between a marine elasmobranch and several teleosts for red muscle (Table 3).

Gluconeogenesis

Gluconeogenic capacity as determined by FBPase activity, a gluconeogenic enzyme responsible for the conversion of glucose-6-phosphate to glucose, was highest in the liver and kidney. Heart showed the lowest FBPase activity.

Amino Acid Metabolism

GDH, AlaAT, and AspAT activities were detected in all tissues studied. GDH, an enzyme which catalyzes the oxidative deamination of glutamate in the formation of α-ketoglutarate, had highest activity in the kidney followed by the red muscle. AlaAT, an enzyme which transaminates alanine directly to produce pyruvate, was most active in the liver and least active in the heart. However, AspAT, an enzyme which transaminates aspartate to produce oxaloacetate, was more active than AlaAT in all tissues, the red muscle having the highest activity with the brain having the least.

Discussion

Lipid Metabolism

Based on HOAD activity, lipid oxidation occurs in the liver as well as all extrahepatic tissues sampled from lake sturgeon. HOAD activity was highest in red muscle and heart in agreement with studies of several teleosts (Ewart and Dreidzic 1987; Moyes et al. 1989). This contrasts with the situation in freshwater and marine elasmobranchs which appear to have a very low capacity to oxidize fatty acids extrahepatically (Moon and Mommsen 1987; Singer and Ballantyne 1989). The extent of oxidation of specific fatty acids can also be assessed by measuring the activity of various carnitine acyl transferases. Carnitine acyl transferases catalyze a rate-limiting step in fatty acid oxidation in mammals; long chain fatty acids that are mobilized from depot triglycerides are esterified to L-carnitine and then transferred by acyltransferases into the mitochondrial matrix where oxidation can occur (see McGarry and Foster 1980, for review). In fish, carnitine dependent oxidation of long and medium chain fatty acids appears to be predominant and more important than carnitine independent fatty acid oxidation (Bilinski and Jonas 1970; Ballantyne et al. 1989). Two forms of carnitine acyl transferases are commonly measured: (1) CPT specific for long chain fatty acyl carnitines and (2) COT specific for medium chain acyl carnitines. In the present study palmitoyl-CoA (C:16) and octanoyl-CoA (C:8) were used as substrates to assess the levels of CPT and COT, respectively.

CPT activity was detectable but very low in brain, red muscle, and heart of the lake sturgeon. These results are in general agreement with those observed in tissues of elasmobranchs (Zammit and Newsholme 1979; Singer and Ballantyne 1989). Dreidzic and Hart (1984) showed that elasmobranch hearts unlike teleost hearts were unable to use palmitate as fuel for work but relied on ketone bodies as a fatty acid substitute. This contrasts with the studies of Moyes et al. (1986) and Ballantyne and Moon (1986) who reported the

oxidation of a wide range of acyl carnitines by isolated liver mitochondria of the marine elasmobranch *R. erinacea*, indicating the presence of CPT and COT in this tissue. Based on CPT activities, the capacity for lipid oxidation of long chain saturated acyl carnitines in extrahepatic tissues of sturgeon appears to lie between that of the elasmobranch and teleost models (Zammit and Newsholme 1979; Dreidzic and Hart 1984).

COT, the enzyme that appears to catalyze transfer of medium chain (8:0) fatty acids into the mitochondria, is detectable in all tissues although levels are low. This suggests that octanoyl carnitine (8:0) may be more important as a substrate for β-oxidation compared with long chain acyl carnitines. However, Sidell et al. (1987) have suggested that CPT may utilize fatty acids other than palmitate and thus CPT activities underestimate the true potential for carnitine dependent fatty acid oxidation. It is known that fish can utilize a broader range of fatty acid chain lengths than most mammals (see Henderson and Tocher 1987 for review). Murata and Higashi (1979) demonstrated that fatty acids such as 18:1,*n*9 and 16:1 were oxidized at much higher rates than 16:0 by mitochondria from dark muscle of carp. Likewise in the lake sturgeon, other longer chain acyl carnitines such as oleoyl carnitine (18:1,*n*9) may be a preferred substrate of carnitine dependent oxidation over palmitoyl carnitine (16:0) based upon levels of oleic acid (18:1,*n*9) which amount to 45% of the total NEFA content of plasma compared with 18% for palmitic acid (16:0). Future studies of the lipid catabolism of sturgeon need to be focused on the importance of mono-unsaturated fatty acids as metabolic substrates.

Based on the present study, low levels of CPT and COT in certain sturgeon tissues compared with those of teleost fishes may be due to: (a) substantial carnitine independent oxidation of certain fatty acids as has been observed in insects (Stevenson 1968; Chamberlin 1987) and lake trout liver mitochondria at low temperatures (Ballantyne et al. 1989), or (b) carnitine-dependent oxidation of unsaturated fatty acids (Henderson and Tocher 1987).

The relatively low level of lipid oxidation in sturgeon tissues appears to be supported by studies of digestive enzymes in sturgeon. Sturgeon have been classified as primarily carnivorous fish (Semakula and Larkin 1968; Scott and Crossman 1973; Houston 1987; Muir et al. 1988) and the adult sturgeon digestive tract contains high levels of trypsin and pepsin for protein digestion but very low levels of lipase for lipid digestion (Buddington 1985; Buddington and Christofferson 1985; Buddington and Doroshov 1986a,b). By contrast, larval sturgeon have been shown to have extremely high levels of digestive lipase which enables them to take advantage of the high lipid content of their diet, mainly cladocerans (Tessier and Goulden 1982). The preferred diet of adult white sturgeon *Acipenser transmontanus* consists of amphipods, which contain much lower levels of lipid (Buddington and Doroshov 1986a,b; Muir et al. 1988). It is known that inclusion of lipid in the diet at levels >15% causes liver dysfunction and excessive fat deposition in juvenile white sturgeon (Buddington and Doroshov 1986a). Future investigations into larval metabolic enzymes may confirm high HOAD and CPT activities which would complement high lipase activities. It is very likely that diets with high fat content may be important to larval sturgeon growth. Particular attention should be paid to the fatty acid composition of diets used in culture operations, with emphasis placed upon monoenes, such as oleic acid (18:1, *n*9), which have been shown to be a major constituent of plasma NEFAs in adult lake sturgeon.

Malic enzyme activities are highest in the liver and low extrahepatically in contrast with the inverse situation in the marine elasmobranch *R. erinacea* (Moon and Mommsen 1987). High malic enzyme activities in the liver, for generation of NADPH required for fatty acid synthesis, are in general agreement with studies conducted on teleosts in which it has been shown that the liver is the most lipogenic tissue (Henderson and Tocher 1987).

Plasma Nonesterified Fatty Acids (NEFAs)

Plasma NEFAs are the most metabolically active form of lipid in the blood of vertebrates and indicate the extent to which fatty acids are utilized as a source of fuel (Henderson and Tocher 1987). In most species of fish, plasma NEFAs represents the mobilized fatty acids from liver and/or adipose tissue through the action of various lipases. Most studies conducted to date on the lipid composition in fish have focused on the fatty acid composition of selected tissues such as muscle or the entire animal (see Greene and Selivonchick 1987 for review). These studies have shown that most fish contain high levels of long chain unsaturated fatty acids, including unsaturated fats of the *n*3 configuration, which may have health benefits to man (Kinsella 1987). However, such studies do not include how important these fats are to the fish itself as metabolic substrates and whether there is a preference for chain length or state of unsaturation for β-oxidation. Thus, precise determination of individual NEFAs in fish plasma is helpful for understanding the turnover (mobilization for oxidation or as eicosanoid precursors) of different chain length fatty acids.

A few studies have measured the total NEFA content in fish plasma using the available, often inaccurate, colourimetric assays (Larrson and Fange 1977; Zammit and Newsholme 1979; Moon 1983; Black and Love 1986). The colourimetric methods frequently employed are not suitable for measurement of individual fatty acids and furthermore, may underestimate total plasma NEFAs. The method employed in the present study allows quantitative determination of individual NEFAs in plasma in a simple one-step reaction (Lepage and Roy 1988). The present study is the first investigation of plasma NEFAs in fish using this effective and more efficient method. Fellows et al. (1980) have measured individual NEFAs in plasma of fish, using the method of Folch et al. (1957) and the analysis of individual NEFAs by a combination of thin-layer and gas-liquid chromatography. The total plasma NEFAs as measured by the method of Lepage and Roy (1988) was more accurate as checked in our laboratory and 2.7-fold higher than was obtained by the method of Folch et al. (1957). Total plasma NEFA levels and fatty acid profiles obtained with the method of Lepage and Roy (1988) in plasma of other fish species (T. D. Singer et al. unpubl. data) suggest that NEFA levels reported so far using other methods are not indicative of true plasma NEFA levels. These include the very low levels of NEFAs in the plasma of fish surveyed by Fellows et al. (1980), e.g. a value of 0.26 ± 0.03 μmol/mL in rainbow trout. NEFA levels in teleost plasma within species appears to vary widely with the method employed, e.g. from 20 μmol/mL in cod *Gadus morhua* (Black and Love 1986) as determined by the colourimetric method of Duncombe (1963) to 1.283 ± 0.173 μol/mL in the same species (Larsson and Fange 1977) by a different colourimetric method (Laurell and Tibbling 1967).

Despite the problems described above, plasma NEFA levels determined using the same method can be compared. Zammit and Newsholme (1979), using the colourimetric assay of Duncombe (1963), surveyed NEFAs in teleosts and elasmobranchs and found that teleosts had 10-fold higher concentrations of plasma NEFAs than did elasmobranchs. A similar trend was apparent in two other surveys of NEFAs in plasma including elasmobranchs and teleosts (Larsson and Fange 1977; Fellows et al. 1980). Comparing plasma NEFAs levels using the method of Lepage and Roy (1988) lake charr *Salvelinus namaycush* and Arctic charr *S. alpinus* have total plasma NEFAs levels two to three times that of the lake sturgeon (T. D. Singer et al. unpubl. data) thereby suggesting that the lake sturgeon has plasma NEFAs levels intermediate between elasmobranchs and teleosts. However, the percentages of *n*9 NEFAs in the plasma are twice those of other fish examined (T. D. Singer et al. unpubl. data) indicating that oleic acid (18:1,*n*9) may be a preferred fuel for β-oxidation. This high concentration of monoenes in the plasma NEFAs may reflect the composition in depot triglycerides although these were not measured in the present study. Unusually high amounts of monoenoic fatty acids have been found in elasmobranch livers (Malins et al. 1965) suggesting that monoenes, such as oleic acid (18:1,*n*9), may be a preferred storage form of fatty acid in primitive fishes. The high levels of long chain fatty acids (AA, 20:4*n*6, EPA, 20:5*n*3, and DHA, 22:6*n*3) in the plasma may indicate a high turnover of eicosanoids in the sturgeon. A review by Bell et al. (1986) indicates that such fatty acids may be used in fish as they are in mammals, for metabolic conversion to eicosanoids.

The striking difference in total NEFAs in the plasma of elasmobranchs compared with teleost fish may be a direct consequence of the absence of serum albumin. Albumin is known to transport free fatty acids mobilized from lipid reserves in mammals. Recently an albumin-like protein capable of binding and transporting free fatty acids has been found to exist in the plasma of salmonids (Davidson et al. 1988). A survey of plasma fatty acid binding proteins in elasmobranchs and teleosts by Fellows and Hird (1981) revealed their absence in elasmobranchs. The levels of fatty acids reported here (>2mM) far exceed the solubility of long chain fatty acids (Windholz 1983). Thus it is likely that the lake sturgeon does possess an albumin-like fatty acid carrier protein and like the teleosts (Zammit and Newsholme 1979) has developed a specific lipid storage tissue and the ability to mobilize and transport NEFAs to other tissues.

Ketone Body Metabolism

The lake sturgeon has low but detectable levels of beta-hydroxybuyrate dehydrogenase in all tissues. This contrasts with the total absence of beta-hydroxybutyrate dehydrogenase in all teleosts (Zammit and Newsholme 1979; Dreidzic and Hart 1984; T. D. Singer et al. unpubl. data). The ability to use β-hydroxybutyrate may be a primitive feature retained from ancient vertebrate ancestors of both elasmobranchs and sturgeon but subsequently lost in the teleost fishes. The extrahepatic tissues of elasmobranchs rely on ketone bodies generated in the liver from lipid stores as an important fuel substitute for fatty acids. SKT levels in lake sturgeon are also similar to those reported for the marine elasmobranch *R. erinacea* in the tissues examined (Moon and Mommsen 1987). The red muscle and heart have the highest level of SKT, suggesting that ketone bodies play an important role in the oxidative metabolism of these tissues. Levels of ACoAT are lower than those reported for both teleosts and elasmobranchs (Beis et al. 1980; Singer and Ballantyne 1989).

Oxidative Metabolism

Overall levels of CS were lower than most teleosts studied to date (Suarez et al. 1986; Ewart and Dreidzic 1987; Sidell et al. 1987; Moyes et al. 1989). CS levels are comparable with the marine elasmobranch *R. erinacea* (Moon and Mommsen 1987). Active oxygen consumption rates are 5.5 times higher in a salmonid (Brett 1972) compared with a white sturgeon of similar weight (Burggren 1978). This reflects the CS levels found in the heart of salmonids by Ewart and Dreidzic (1987) which are four times higher than those in the sturgeon. As well, Burggren et al. (1979) have measured the weight specific gill areas of lamellar blood channels in white sturgeon and found them to be lower that that of other fish reflecting a low activity level and metabolic rate in sturgeon. Thus the maximal aerobic capacity of the sturgeon is considerably lower than most telosts studied to date which may be a reflection of the sturgeon's benthic lifestyle.

Glycolysis

The highest HK levels are found in the kidney followed closely by heart and brain. Brain HK activity is low. Typically the brain in most vertebrates has the HK activity of all tissues since it relies heavily on glucose for its energy requirements. HK and PFK levels are low in all tissues compared with values reported in teleosts (Moon 1983; Ewart and Driedzic 1987; Sidell et al. 1987). HK levels are similar to those found in the marine elasmobranch *R. erinacea* by Moon and Mommsen (1987); PFK levels are lower. Surprisingly the highest PFK levels are found in the red muscle although these are low in comparison to those in teleost fish. The low levels of these enzymes suggest that carbohydrates are not the preferred fuel in most tissues.

The lake sturgeon has lower anaerobic capacity based upon LDH:CS ratios in heart compared with all other species of fish while red muscle was intermediate between a marine elasmobranch and several teleosts (Table 3). This finding contrasts with the known anoxia tolerance of sturgeon. A related species, the white sturgeon is frequently found in deoxygenated benthic regions of lakes, in ice-covered waters and stagnant regions of estuaries where an anaerobic metabolic strategy would be beneficial. Burggren et al. (1985) have suggested that the white sturgeon responded to hypoxic situations by a form of metabolic depression. Burggren and Randall (1978) investigated the reaction of 1-kg white sturgeon to low dissolved oxygen environment and provided evidence of a metabolic decrease under mild hypoxia. They also discovered that there was no repayment of the oxygen debt even after severe hypoxic exposure, a response incompatible with excessive lactate or succinate accumulation. Cech et al. (1984) found growth rates of 1–6-g juvenile white sturgeon to be significantly reduced under hypoxia, indicating a depression of metabolic rate. In contrast, a study conducted by Ruer et al. (1987) to determine the effect of population density and hypoxia on routine metabolism in white sturgeon showed no differences in metabolic rates for 1.73- to 2.17-kg individuals under normoxic and hypoxic conditions. Based on the low LDH:CS ratios, and its known anoxia tolerance, the lake sturgeon may utilize a different metabolic strategy to survive hypoxia.

A small number of fish species, including goldfish *Carassius auratus*, crucian carp *C. carassius*, and bitterling *Rhodeus amarus*, rely on the anaerobic production of ethanol, ammonia, and CO_2. These animals much like the sturgeon are very resistant to anoxia and are able to survive extreme seasonal conditions (Van den Thillart et al. 1989). Unlike the sturgeon, the goldfish maintains typically high LDH levels in heart and red muscle (Van den Thillart and Smit 1984). The sturgeon may rely on an inherently low metabolic rate and alternative fermentations of carbohydrates to survive anoxic conditions and avoid lactate acidosis. Further studies are required to determine the presence or absence of alternative fermentation pathways in the extrahepatic tissues, especially red muscle, of the sturgeon. The existence of a metabolic depression during anoxia also requires further investigation.

Gluconeogenesis

Elevated levels of FBPase activity in the liver and kidney compared with all other tissues indicate these tissues are the major gluconeogenic sites as occurs in marine elasmobranchs (Moon and Mommsen 1987), freshwater elasmobranchs (Singer and Ballantyne 1989), as well as many teleosts (see Suarez and Mommsen 1987 for review). As with other carnivorous fish amino acids may be the preferred metabolic precursors for gluconeogenesis in sturgeon.

Amino Acid Metabolism

Based on the moderate levels of GDH, AlaAT, and AspAT the lake sturgeon can utilize amino acids as oxidative substrates. The potential to utilize glutamate and alanine is highest in the kidney compared with other tissues. Comparison to a teleost, the goldfish, indicates similar enzyme profiles with highest activities in the red muscle and lowest in the liver (Van Waarde 1981). This trend is not observed with the other enzymes of amino acid metabolism. A high reliance on amino acids is reflected by the digestive enzyme complement in sturgeon; high in trypsin and pepsin secretions in the adult (Buddington 1985; Buddington and Doroshov 1986a,b), corresponding to adult sturgeon carnivorous habits (Semakula and Larkin 1968; Scott and Crossman 1973; Houston 1987; Muir et al. 1988).

Evolutionary Considerations

Based upon many morphological characteristics sturgeons have been placed in an evolutionary intermediate position between elasmobranchs and teleosts. They have been separated from the main line of teleostean evolution for at least 200 million yr (McEnroe and Cech 1987). The present study indicates the metabolic organization of the sturgeons is also intermediate between the "primitive" elasmobranch and the "advanced" teleost. This stage is especially evident in the reduced lipid catabolism in extrahepatic tissues of the sturgeon similar to that observed in elasmobranchs. Levels of NEFAs found in the plasma of the lake sturgeon suggest that the turnover (mobilization and oxidation) of fatty acids is intermediate between the very low NEFA levels in elasmobranchs and the 10-fold higher levels found in teleosts. In addition, the sturgeon, much like the elasmobranch, has detectable levels of beta-hydroxybutryate dehydrogenase, distinguishing it from all teleosts examined to date.

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Influence of Prey Abundance on Species and Size Selection by Young Yellow Perch (*Perca flavescens*)

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Prey selection by young yellow perch (*Perca flavescens*) (22–62 mm TL) was measured during 11 experiments over two summers, 1982–83. For each experiment fish were offered different densities of the same mixture of zooplankton from Oneida Lake, NY. As density of prey increased, several measures of selectivity for *Daphnia* either remained unchanged or declined, while all measures of selectivity for calanoids and cyclopoids increased. At all densities small to mid-sized *Daphnia* were preferred to large *Daphnia*. Reanalyses of other studies suggests this may be the general pattern for young planktivores, despite the contrary predictions of several models of prey selection. For young planktivores, differences in the nutritional quality of zooplankton prey, including resultant growth efficiency and speed of digestion, can account for these results.

Le choix de proies des jeunes perchaudes (*Perca flavescens*) (longueur totale 22–62 mm) a été mesuré au cours de onze expériences réalisées durant les étés de 1982 et de 1983. Pour chaque expérience, on a offert aux poissons diverses concentrations du même mélange de zooplancton provenant du lac Oneida dans l'état de New York. À mesure qu'augmentait la concentration de proies, plusieurs mesures de la sélectivité de *Daphnia* sont demeurées inchangées ou ont diminué, alors que toutes les mesures de sélectivité des calanidés et des cyclopidés ont augmenté. Pour toutes les concentrations, les petites et moyennes *Daphnia* ont été préférées aux plus grosses. Les analyses d'autres études semblent indiquer que cela est généralement ainsi dans le cas des jeunes planctonivores, malgré les prédictions contraires faites par plusieurs modèles de sélection des proies. Ainsi, dans le cas des jeunes planctonivores, les différences de qualité nutritive des proies (zooplancton), y compris la vitesse de digestion et la croissance qui en découle, pourraient expliquer ces résultats.

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Studies with fasted planktivores feeding briefly in a high density of prey have shown that large zooplankton provide the highest reward rate ($g \cdot t^{-1}$) (Werner 1974; Mittelbach 1981; Li et al. 1985; Mills et al. 1984; Confer and O'Bryan 1989). However, young planktivores sometimes select for smaller prey that do not provide the maximum short-term reward (Furnass 1979; Hansen and Wahl 1981; Bence and Murdoch 1986). Both field and laboratory studies showed that young yellow perch (*Perca flavescens*) selected more strongly for copepods and mid-sized *Daphnia* (0.8–1.7 mm) than for larger *Daphnia* (Mills et al. 1984, 1986).

Nutritional differences among available prey may help explain selection for smaller prey. Differences in growth efficiency by planktivores on diets of different prey have been reported for *Salmo salar* (Wankowski and Thorpe 1979), *Sarotherodon galilaeum* (Gophen 1980), and young yellow perch (Confer and Lake 1987; Mills et al. 1989). Food pellets of different composition can have quite different specific dynamic action (Schalles and Wissing 1976). Field samples have shown that growth of young yellow perch during mid-summer correlated with the biomass of available small (<1.1 mm) and medium (1.2–1.7 mm) *Daphnia pulex* but not with larger *Daphnia pulex* (Mills et al. 1989).

Mills et al. (1986) suggested that digestive factors might account for a preference for smaller prey as shown by young yellow perch when feeding continuously. Examining this idea, Confer and O'Bryan (1989) determined reward rates and prey preference during short-term and long-term feeding for fish maintained in a high density of prey. Initially large prey provided the greatest reward rate and were preferred by young rainbow trout (*Oncorhynchus mykiss*) and yellow perch. However, during long-term feeding the smallest prey, *Diaptomus*, was egested most rapidly, provided the greatest or nearly the greatest reward rate of several prey types, and was preferred by both fishes.

In this study we analyze the influence of prey density on prey selection by yellow perch allowed to feed for 1 h at natural prey densities. Herein, we test if prey density influenced the species

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and species-specific size selection by young yellow perch. We specifically test if preference changed from *Daphnia* to copepods at higher prey densities as was predicted by Confer and O'Bryan (1989). The data analyzed in this study was described previously with a stochastic model that showed: the size of all prey eaten decreased as light intensity increased, the mean size of all prey eaten was not influenced by prey density, and larger fish selected more strongly for *Daphnia* and less strongly for copepods (Mills et al. 1986).

Methods

Procedures

Laboratory experiments were conducted from June through September of 1982 and 1983. Young yellow perch were seined from near shore of Oneida Lake, NY, on the morning of each experiment and transferred to a holding tank. These fish were provided a continuous supply of *Daphnia* for 2–4 h. Fish were then sorted by size with 7–20 fish transferred to each experimental tank which contained 500–900 L of filtered lake water. To minimize prey depletion during the experiment, we used fewer of the larger fish in late summer and larger water volumes for low density trials. Fish were fasted in the experimental tank for 1 h to standardize hunger and acclimate the fish. During each trial, the fish fed in an undisturbed room for 1 h after the addition of zooplankton after which they were anesthetized with tricaine (ms-222) and preserved in 10% formalin.

Temperatures in the experimental tanks matched lake temperatures, and light intensities were between 200–400 lux. Zooplankton were collected by horizontal tow from Oneida Lake on the day of the experiment using a 153-μm mesh net, and maintained alive in a cooled, 60-L stock aquarium. The density of zooplankton in this sample and the water volume of each experimental tank was measured. A subsample was taken from the stock aquarium and a measured volume was added to each experimental tank to produce the desired zooplankton density for each trial.

Zooplankton Enumeration

Our procedures for enumeration of zooplankton from fish guts and environmental samples have been described (Mills et al. 1984, 1986) with details of the computerized process in Mills and Confer (1986). Zooplankton were grouped into the following categories: *Daphnia* (including *D. pulex* [Leydig] and *D. galeata mendotae* [Birge]); calanoids (including all copepodites of *Diaptomus minutus* [Lilljeborg], *Diaptomus oregonesis* [Lilljeborg], and *Epischura lacustris* [Forbes]); cyclopoids (including all copepodites of *Cyclops bicuspidatus* [Claus] and *Mesocyclops edax* [Forbes]); nauplii; *Eubosmina; Chydorus;* and *Sida*.

Data Analyses

An experiment is defined as two to four trials on one date. Each trial of one experiment tested selection at a different prey density. All trials of one experiment were provided with prey from the same stock aquarium. Thus, the proportion and size distribution for each prey type were the same for all trials of one experiment. All fish for one experiment were collected in one place at one time. Within an experiment the range of fish size generally was less than 10% with a maximum of 14%. Among all experiments, which were conducted over 4 mo for each of two summers, large differences in fish size and prey proportions occurred. Changes in species and species-specific size preference that could be consistent among trials of one experiment might be obscured by pooling data for all experiments. Consequently, the changes in species and species-specific size preference were determined by comparisons among the trials within one experiment.

For all trials we determined the proportion of a prey-size category in the diet, r_i. For graphic clarity this proportion is presented as an adjusted forage ration (AFR) of $r_i/p_i - 1$, where p_i is the proportion of a prey-size category in the experimental tank. An AFR of -1 represents complete avoidance while 0 represents an equal proportion eaten and available. Since p_i was constant among the trials of one experiment, changes in the AFR are due to changes in r_i. An AFR was calculated for each 0.2-mm size increment of each prey type.

Laboratory and Field Comparisons

Mills et al. (1984) showed that prey selection under these laboratory conditions and in the lake were very similar. They found that laboratory and lake fish had the same relationship between fish size and species preference. Mills et al. (1986) pooled the data used in this current study and found that laboratory and lake fish had the same relationship between: light intensity and mean size of all prey eaten; prey density and mean size of all prey eaten, and once again; fish size and species preference.

Results

A total of 11 experiments were conducted during 1982 and 1983, including two experiments from each of two dates derived by splitting the fish into two size groups. Prey densities used during 1982 ranged from 10 to 80 crustacea plus rotifers $\cdot L^{-1}$, encompassing the commonly occurring densities in Oneida Lake. A wider range of prey densities, 5–500 $\cdot L^{-1}$, was used in 1983 to test fish under circumstances that might elicit a stronger, and more detectable response to changes in prey density. In all trials, *Daphnia*, calanoids, and cyclopoids were the most abundant prey and were most frequently eaten (Table 1). Other prey groups were rarely eaten.

The abundance of prey in the low density trials was thought to be less than optimum for fish growth for two reasons. First, the lower densities corresponded to densities of zooplankton that limit fish growth in Oneida Lake (Mills and Forney 1981). Second, the number of prey consumed per fish increased from a lower density trial to the next higher density trial of an experiment in 19 of 25 comparisons (Table 1).

Prey depletion was never significant for trials using the medium and higher prey density as only 0.04 to 2% of the available prey were consumed. Among low density trials, prey depletion occasionally was severe for strongly selected size-types. For the three low-density trials most affected, depletion of the most selected type-size categories of prey was estimated to be 113, 76, and 52%. The estimated depletion of 113% probably results from error in sub-sampling the stock zooplankton. The degree of depletion for all other prey declined rapidly from these values. Since overall there were over 1000 prey categories from 37 trials of 11 experiments, prey depletion generally was not a significant factor.

TABLE 1. Synopsis of prey available and eaten. *Eubosmina*, *Chydorus*, *Sida*, nauplii, and rotifers occurred but collectively never exceeded 2.1% of the fish diet for any trial and are not presented separately in the table. However, these prey were included in calculations. Abbreviations: Fish^{-1} for number of prey eaten; Env for values from the subsample of the stock zooplankton aquarium.

				Prey sample percentage						Prey sample size	
				Cyclopoids		*Calanoids*		*Daphnia*			
Date	Number of Fish	Fish size range (TL mm)	Prey density (#/L)	Env.	Fish^{-1}	Env.	Fish^{-1}	Env.	Fish^{-1}	Env.	Fish^{-1}
061882	18	22–25	10	7	16	4	0	85	81	1005	6.5
	19		20		1		3		96		8.9
	25		40		4		2		98		11.3
	24		80		1		2		97		15.0
071382	14	30–34	10	12	8	42	20	42	72	1028	47.9
	15		20		7		29		64		38.3
	7		40		48		37		15		69.7
	6	35–39	10		9		20		71		35.2
	7		20		18		48		34		55.3
	7		40		26		39		35		62.3
	14		80		28		20		52		55.2
072782	13	35–40	10	30	28	44	57	7	14	705	55.2
	7		35		28		48		24		83.0
	6		80		15		53		32		68.2
	4	41–47	10		27		49		18		25.0
	9		35		24		48		27		66.0
	7		80		20		63		17		115.4
081082	9	42–48	10	29	9	23	54	7	32	701	40.9
	6		35		15		64		18		45.8
	9		80		27		63		9		46.6
082482	4	54–62	35	11	18	19	40	11	31	593	30.7
	4		80		10		41		42		63.3
062383	9	26–30	16	10	2	54	86	24	12	520	23.1
	13		64		3		82		17		31.8
	16		145		4		84		12		52.3
071583	6	36–45	5	10	0	33	10	36	90	505	28.2
	11		50		1		22		78		34.6
	17		200		2		55		44		39.1
	6		500		2		31		31		71.3
072083	9	45–54	50	10	1	51	3	38	96	501	17.2
	10		200		1		4		95		10.4
	8		500		1		20		79		13.4
080283	7	45–57	5	11	1	38	1	38	98	534	28.0
	6		50		1		4		95		18.8
	9		200		1		33		65		32.2
	6		500		1		1		55		27.3

Species Preferences

Young yellow perch (22–62 mm TL) virtually ignored the smallest prey, such as *Chydorus*, nauplii, and rotifers. Only the smaller yellow perch had a positive AFR for the smallest Daphnia of 0.5–0.7 mm (Fig. 1). All sizes of perch at all prey densities had low to negative AFR for the largest Daphnia (>2.1 mm) (Figs. 1 and 2). All sizes of yellow perch usually had positive AFR for the largest copepods, which were smaller than the largest *Daphnia*. Of six experiments with smaller fish (<43 mm TL) an AFR >4 occurred five times with *Daphnia* and calanoids and four times with cyclopoids. Of five experiments with larger fish, an AFR >4 always occurred for *Daphnia* but occurred three times for calanoids and only once for cyclopoids. During 1982, *Daphnia* decreased greatly in the lake during the summer and usually comprised less than 50% of the prey eaten in experiments. During 1983, *Daphnia* remained abundant in the lake throughout the summer, and usually comprised considerably more than 50% of the eaten prey while cyclopoids were too infrequent for analyses (Table 1).

Prey Type Selection and Prey Density

The maximum AFR for a prey type is one indicator of selectivity. For each of the three major prey types (calanoids, cyclopoids, and *Daphnia*), the maximum AFR for any size at each lower density trial was compared with the maximum value at each higher density trial of the same experiment. For *Daphnia*, increases and decreases in the maximum AFR when summed for both years occurred with virtually identical frequency (Table 2). For both calanoids and cyclopoids, the maximum AFR generally increased as prey density increased. For calanoids the increase in AFR was statistically significant in 1982

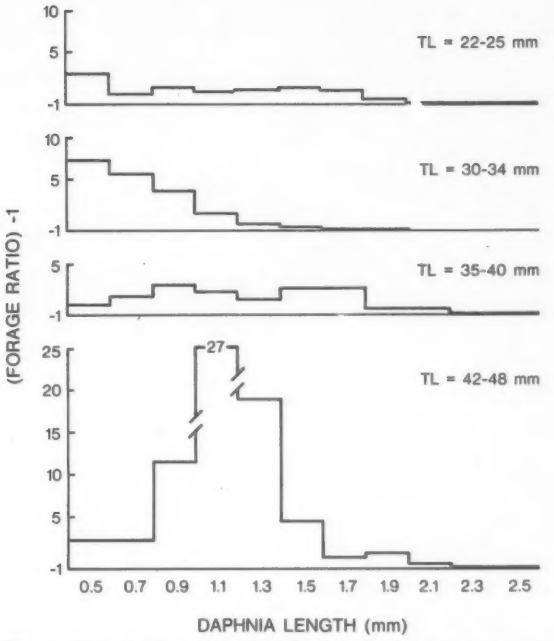

FIG. 1. Size-specific selection for *Daphnia* by four sizes of young yellow perch at prey densities of $10 \cdot L^{-1}$. Data from experiments on 18 June, 13, and 27 July, and 10 August 1982.

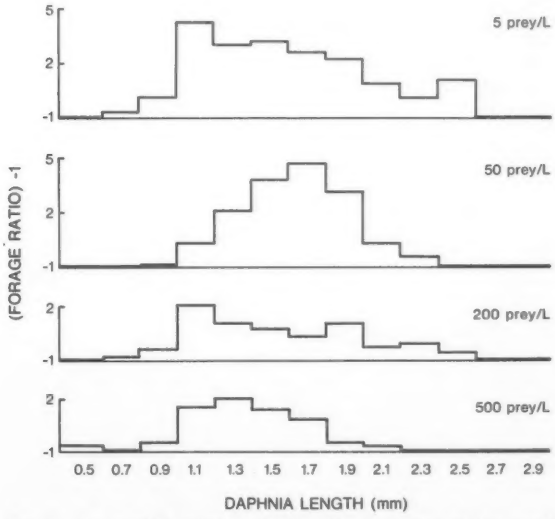

FIG. 2. Size-specific selection for *Daphnia* by young yellow perch (36–45 mm TL) offered the same species-size proportions of a mixture of prey species at different densities on 15 July 1983. These results show the typical pattern of selection for mid-sized *Daphnia* and that the size range with a positive AFR (see text) and the maximum AFR generally declined with increasing prey density.

and when summed for both years (Table 2). For cyclopoids the AFR increased in 64% of the comparisons in 1982 but this was not statistically significant.

Another measure of selectivity is the range of sizes with positive AFR. Pooled for two years, *Daphnia* showed a significant decrease in the number of sizes with a positive AFR as prey density increased (Table 2). Calanoids showed a trend for a wider range of sizes having a positive AFR as prey density increased for both years which was not significant. Cyclopoid data for 1982 showed a significant increase in size range, and the combined results for copepods, i.e. cyclopoids and calanoids, showed an increase in size range in 27 of 36 (75%) comparisons which was highly significant ($G = 9.42$, $p < 0.01$).

The proportion of *Daphnia* in the diet decreased in 26 of 43 comparisons, but this trend was not significant. Copepods, which comprised most of the rest of the diet, showed the reciprocal trend.

Prey Size Selectivity and Prey Density

For 1982 the mean size eaten increased in 49 out of 72 (68%) of the comparisons from a lower to higher density trial ($G = 9.60$, $p < 0.01$). This increase was primarily due to the fish response to copepods whose mean size increased in 74% of the comparisons while *Daphnia* mean size increased in only 58% of the comparisons. As prey density increased, two contrary tendencies occurred for size selection during 1982. Within a taxon, the fish ate proportionally more of the larger prey, particularly larger copepods. Among taxa the fish ate proportionally more of the smaller prey types, copepods, and fewer of the larger prey type, *Daphnia*. The resultant increase in mean size of all eaten prey for 15 of 25 comparisons was not significant ($G = 1.01$, $p > 0.1$).

For 1983 prey selection was tested with a wider range of prey densities in the expectation that this would stimulate more decisive results. However, during the summer *Daphnia* remained abundant and the mean size eaten increased in less than half (17 of 41) of the comparisons which is not statistically significant. The significant increase during 1982 was largely due to the response to copepods, and the contrary result for 1983 may be due to the sustained abundance of *Daphnia* during the later year. Combining both years, the mean size within taxa increased in 58% of the comparisons and the mean size of all prey increased in 53%, neither of which is statistically significant.

Discussion

Several trends in these results are consistent for both years despite yearly differences in proportions of prey available and some differences in prey consumed. The range of sizes of *Daphnia* with a positive AFR decreased both years and the summed change was significant. The proportion of *Daphnia* in the diet tended to decrease both years, and the AFR for *Daphnia* failed to increase significantly either year. Conversely, for calanoids and cyclopoids both maximum AFR and the range of sizes with positive AFR increased in all comparisons with a statistically significant increase for results summed for both years. Despite these trends, *Daphnia* are the largest of these prey and provide the greatest reward rate ($g \cdot t^{-1}$) for hungry, young yellow perch feeding for a few minutes in a high density of prey (Confer and O'Bryan 1989). Encounter frequency models predict planktivores should select most strongly for larger prey, and optimal foraging models predict that the degree of selectivity for larger prey should increase with increasing prey density (e.g. Werner and Hall 1974; Stephens and Krebs 1986). These models assume that all prey types are nutritionally equal and that ingestion rates determined with fasted fish feeding briefly in a swarm of prey is an appropriate measure of prey rank.

TABLE 2. Changes in maximum adjusted forage ratios (AFR) and in range of sizes with a positive AFR for *Daphnia*, calanoids, and cyclopoids shown by age 0 yellow perch. Results for each taxon were tabulated in terms of increase (L) or decreased (D) from a lower dnesity trial to a higher density trial of one experiment and summed for all experiments. The corrected *G*-value was calculated (after Sokal and Rohlf 1981) for comparisons with more than 10 non-tie observations. *indicates $P<0.05$.

	Adjusted forage ratio									Prey size range								
	Daphnia			*Calanoids*			*Cyclopoids*			*Daphnia*			*Calanoids*			*Cyclopoids*		
Year	I	D	G	I	D	G	I	D	G	I	D	G	I	D	G	I	D	G
1982	14	11	0.35	14	5	4.79*	16	9	2.00	6	14	3.40	10	6	1.00	10	1	11.05*
1983	5	10	1.71	7	2	—	—	—	—	5	9	1.15	7	2	—	—	—	—
Total	19	21	0.10	21	7	7.92*	—	—	—	11	23	4.46*	17	8	3.40	—	—	—

For young yellow perch there is evidence that both assumptions are wrong. Equal rations by weight of different species and sizes of prey produced different growth efficiencies for young yellow perch (Confer and Lake 1987). Further, growth of young yellow perch in early to mid-summer in Oneida Lake correlated with small and medium but not large *Daphnia* (Mills et al. 1989). At least for young yellow perch, the value of a prey is not proportional to the maximum weight that can be ingested per time by fasted fish feeding briefly in a prey swarm.

Confer and O'Bryan (1989) observed that prey rank and preference changed as gut fullness changed for several young planktivores. Initially *Daphnia* were preferred while during continuous feeding *Diaptomus* were preferred over larger prey. In a swarm of mixed prey types, the young planktivores initially selected for the largest prey and then switched to the much smaller *Diaptomus*. Based on these observations, the authors suggested that young fish would select for prey that provided the maximum ingestion rate at low prey densities, but would select for the prey that were digested more rapidly and provided the greater growth efficiency at higher prey densities. Generally, prey selection observed in this study agreed with those suggestions.

It remains to be determined if young yellow perch are typical of young planktivores. Diet changes with increasing gut fullness for *Gambusia* (Bence and Murdoch 1986) are similar to those we have reported for young yellow perch. Prefed *Gambusia*, compared with fasted fish, attacked a significantly higher proportion of smaller prey, which provided the maximum reward for these small fish. Despite greater selectivity for the optimum prey, the prefed fish had a lower ingestion rate than starved fish which was interpreted as a qualitative failure "to meet the basic goal assumed by optimal diet theory" (Bence and Murdoch 1986). However, prefed fish are incapable of ingesting prey as rapidly as starved fish (Confer and O'Bryan 1989) and it is inappropriate to test for optimal foraging by comparing ingestion rates by prefed fish to rates by fasted fish. Despite the decline in ingestion rate, the greater selectivity for smaller prey by *Gambusia* does seem to qualitatively agree with optimal foraging.

Mittelbach (1981) and Werner et al. (1983) measured the size-specific selectivity by *Lepomis macrochirus* on *Daphnia*, and suggested that the results generally agreed with predictions of the model of optimal foraging described by Werner and Hall (1974). This model predicts that *Daphnia* below a minimum size will not be eaten, and selectivity for *Daphnia* above that size will be equal. Werner et al. (1983) provided size-frequency data for ingested and available *Daphnia*, permitting a reanalysis of size-specific selection by the bluegill. In figure 1 of Werner et al. (1983), the abundance of each *Daphnia* size was weighted by their probability of visual detection following the model of Confer and Blades (1975). In our Fig. 3, AFR were calculated for all sizes of *Daphnia* using the values for the encounter frequency and consumption frequency from Werner et al. (1983). Mittelbach (1981) reported similar trends, but in a less detailed manner. Mittelbach (1981) and Werner et al. (1983) noted that the smaller fish consumed a smaller mean size of prey than the

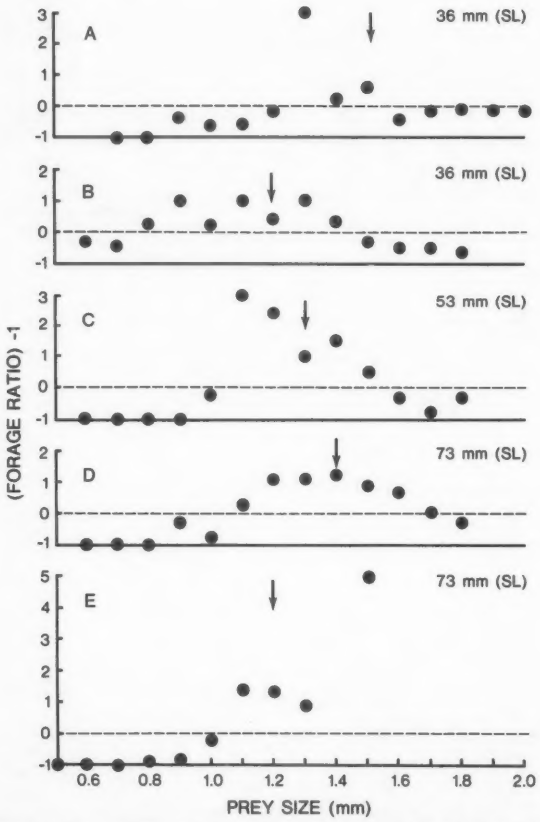

FIG. 3. Size-specific selection for *Daphnia* by bluegill (*Lepomis macrochirus*) recalculated from Werner et al. (1983). AFR determined from the encountered and eaten panels of their figure 1. Arrows for each panel indicate the minimum size of prey that should have been eaten for optimum diet as predicted by their model.

larger fish. They suggested this might be due to a lesser ability of the smaller fish to discriminate prey size before attacking, and/or to the estimated small effect on the fitness of the diet if the smaller prey were eaten by the smaller fish. Li et al. (1985) also suggested that the reduced visual acuity of smaller fish could account for a diet composition that was thought to indicate poor size discrimination. However, analyses of three samples of smaller bluegill (mean TL of 36 or 53 mm) showed a change from complete or nearly complete avoidance to the maximum AFR over a 0.4-mm range of prey size. This is strong indication that smaller bluegill can discriminate between prey that differ by as little as 0.4 mm. Yet, the highest AFR were for prey 0.4 to 0.6 mm smaller than the predicted cutoff size, and over 50% of the prey eaten were smaller than the predicted minimum size for all trials with smaller bluegill (Mittelbach 1981; Werner et al. 1983). Furthermore, their model predicts that larger prey above the minimum cutoff size should be pursued with equal selectivity. Since the estimated encounter frequency for each prey size included an adjustment for relative visibility, all sizes above the cutoff should have the same AFR. To the contrary, values from smaller bluegill declined to circumneutral or negative for *Daphnia* sizes above the predicted minimum.

The size selectivity for *Daphnia* shown by young yellow perch and young bluegill are remarkably similar. During continuous feeding neither of these fishes selected for the prey that provided the maximum ingestion during a brief burst of feeding after fasting. Both fishes showed maximum selectivity for mid-sized *Daphnia* and low to negative values for small and large *Daphnia*. Results for both fish species are compatible with the premise that prey selection by young planktivores is influenced by the nutritional quality of the prey.

Prey selection by young planktivores seems to be quite different from selection by older and larger planktivores (e.g. Wright and O'Brien 1984). Perhaps nutritional factors are more important to young planktivores because their guts and digestive capacity are not fully developed as Confer and Lake (1987) suggested. Even by August young yellow perch, for example, still have poorly differentiated guts with few convolutions. While additional analyses of nutritional effects for young planktivorous fishes are desirable, it is clear that prey selection is influenced by prey quality.

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Hydrogen Peroxide Decay in Waters with Suspended Soils: Evidence for Biologically Mediated Processes

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Hydrogen peroxide decay studies have been conducted in suspensions of several well-characterized soils and in natural water samples. Kinetic and product studies indicated that the decay was biologically-mediated, and could be described by pseudo first-order rate expressions. At an initial H_2O_2 concentration of 0.5 μM, the hydrogen peroxide half-life varied from 1 to 8 h. The decay was inhibited by thermal and chemical sterilization of the soils. Peroxidase activity was inferred in several natural water samples, where the suspended particles catalyzed the oxidation of *p*-anisidine by hydrogen peroxide. The mass spectrum of the major reaction product indicated that it was the dimer, possibly benzoquinone-4-methoxyanil, a product that also was observed from the horseradish peroxidase-catalyzed oxidation of *p*-anisidine by hydrogen peroxide.

Des études sur la décomposition du peroxyde d'hydrogène ont été effectuées dans des suspensions de plusieurs sols bien caractérisés ainsi que dans des échantillons d'eau naturelle. Des études cinétiques et de produits révèlent que la décomposition était biologiquement médiatisée et pouvait être décrite par des expressions de pseudo premier ordre. À une concentration initiale de 0.5 μm de H_2O_2 la demi-vie du peroxyde d'hydrogène variait de 1 à 8 h. La décomposition a été ralentie par la stérilisation thermique et chimique des sols. L'activité de peroxydase a été inférée dans plusieurs échantillons d'eau où les particules en suspension catalysaient l'oxydation de *p*-anisidine par le peroxyde d'hydrogène. Le spectre de masse du principal produit de réaction a révélé qu'il s'agissait du dimère, peut être benzoquinone-4-méthoxyanil, un produit qui a également été observé dans l'oxydation catalysée par la peroxydase de raifort de *p*-anisidine par le peroxyde d'hydrogène.

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Hydrogen peroxide is widely distributed in the hydrosphere (e.g. Cooper et al. 1988). It plays an important role in various environmental redox processes, including oxidations of bisulfite in cloud water (Penkett et al. 1979) or of organic substances in surface waters (Draper and Crosby 1981, 1984; Skurlatov et al. 1983; Zepp et al. 1987b). Recent studies further suggest that H_2O_2 is involved in redox reactions of trace metals such as iron and copper in aquatic environments (Moffett and Zika 1983, 1987a,b). The hydrogen peroxide in fresh waters and sea water is derived from several sources. Of these sources, photochemical formation in the water bodies predominates. Sunlight absorption by dissolved organic matter (DOM) (Baxter and Carey 1983; Cooper and Zika 1983; Cooper et al. 1988, 1989b; Draper and Crosby 1983b; Kockow and Jacob 1986; Sinel'nikov 1971a,b; Sinel'nikov and Liberman 1974; Sinel'nikov and Demina 1974; Zepp et al. 1987a; V. Sturzenegger, and J. Hoigné 1987, Swiss Federal Institute for Water Resources and Water Pollution Control, Duebendorf, Switzerland, pers. comm.), flavins (Mopper and Zika 1987), abiotic particles (Kormann et al. 1988), and algae (Zepp et al. 1987b) all result in its photochemical formation. Hydrogen peroxide also can be produced in the dark through processes mediated by reduced trace metals (Moffett and Zika 1983, 1987a,b) and possibly marine algae (Palinek et al. 1987; Palenik and Morel 1988). External inputs from atmosphere deposition do occur, but are of less importance than these in situ processes (Cooper et al. 1987; Thompson and Zafiriou 1983).

Processes that lead to the decay of H_2O_2 in natural waters are poorly understood. Zepp and co-workers (1987a) have discussed the possible role of algae, which may in part explain the rapid decay of H_2O_2 ($t_{1/2}$ = 1 h) reported for a highly eutrophic water (Draper and Crosby 1983a). In another study, no relationship to chlorophyll *a* was noted in samples obtained from different depths in Lake Ontario and Lake Erie (Cooper et al. 1989a).

In this study, our objectives were to develop rate expressions that describe the decay of hydrogen peroxide in surface fresh waters and to determine the nature of the decay process. We report results indicating that biological processes are the major pathway for the decay of H_2O_2 in aqueous suspensions of well-defined soils and in two natural water samples. Results of the study further demonstrate peroxidase activity in the natural water samples and a soil suspension.

Materials and Methods

Hydrogen peroxide (30%), obtained from J. T. Baker Co. (Analyzed Reagent), was diluted to make a stock solution for

use in these studies. The stock solution was standardized by permanganate titration. *p*-Anisidine (99%) was obtained from Aldrich Chemical Co. and was used as received. Sodium azide, mercuric chloride, and formaldehyde were reagent grade and were used as received. Horseradish peroxidase from Sigma Chemical Co. (210 purpurogallin units/mg, Lot 125F-9640) was used in the anisidine oxidation studies. Twice-distilled water was used in the studies, with the second distillation from alkaline permanganate.

Several soils were selected from a series of well characterized samples (Means et al. 1978). Selected chemical data, sampling locations and a description of the soils, which are identified by number, are summarized in Table 1.

Water samples were collected from the top metre of two small impoundments, Bar-H and Beaver Dam lakes, and from the Middle Oconee River in Athens, Georgia. No sediment settled to the bottom of the collection bottles when the samples were allowed to stand for 24 h. Thus, the samples were used without fractionation within 48 h of the time that they were collected.

Equipment and Analytical Procedures

Hydrogen peroxide was analyzed using the scopoletin/peroxidase method and equipment that is described elsewhere (Cooper et al. 1988; Holm et al. 1987; Kieber and Helz 1986). Calibration curves were obtained for each kinetic system that was investigated. *p*-Anisidine and its oxidation products were analyzed using a Beckman Model 110B high pressure liquid chromatograph equipped with a Kratos Spectroflow 757 variable wavelength uv-visible detector set at 295 nm to detect *p*-anisidine and at 490 nm to detect only the oxidation product. An Absorbosphere HS column (5 μm) with 40–60% acetonitrile-water and flow of 1.5 mL/min was used. The electronic absorption spectra were obtained using a Perkin Elmer Lambda 3 spectrophotometer. The EI mass spectrum of the major anisidine oxidation product was obtained by direct probe on a Finnigan EI/CI mass spectrometer system.

Soil suspensions

Soils (3 to 4 g) were suspended in distilled water (800 mL). They were shaken at 150 rpm in 1-L Erlenmeyer flasks for 48 h at 22°C in the dark. The samples were allowed to settle for 24 h and the top 10 cm was removed so as not to disturb the underlying settled particles. This layer contained the clay fraction (< 2 μm) (Miller and Zepp 1979), which was used in all of the experiments. Once isolated, these clay suspensions were maintained at 22°C in the dark on a shaker table (150 rpm). As mentioned above, the natural water samples were used without the fractionation procedure. Just prior to the kinetic studies (see below), samples were transferred to a shaker/water bath and maintained at 25 ± 0.5°C during the experiments. All samples were adjusted to pH 7.00 (0.01 M phosphate buffer) prior to the addition of H_2O_2. Soil concentrations were determined as described by Miller and Zepp (1979).

The oxygen tension of the soil samples was not measured, but agitation by the shaker/incubator insured equilibrium with atmospheric oxygen.

Kinetic studies: general procedure

The kinetic studies were begun by adding an aliquot of the hydrogen peroxide stock solution to the soil suspension. The initial concentration of hydrogen peroxide in most experiments was 0.5 μM. Periodically, aliquots of the suspensions were

TABLE 1. Chemical composition of selected characterized soils used in this study (Means et al. 1978).

Soil	Description and source
7	Silty clay loam, dark enough to be classified as Mollisol, from near Big Bend Dam on Missouri River, southwest of Pierre, South Dakota Chemical Composition 2.09 % Org-C 3.71 % Fe 6765 Mn ($\mu g \cdot g^{-1}$)
10	Silt loam texture, fairly light in color, eroded loess-derived soil sampled west of junction of Iowa I75 and 37 in central Iowa Chemical Composition 2.10 % Org-C 2.21 % Fe 806 Mn ($\mu g \cdot g^{-1}$)
12	Upland soil taken from area next to a tobacco field near Manchester, Ohio Chemical Composition 2.33 % Org-C 3.05 % Fe 2271 Mn ($\mu g \cdot g^{-1}$)
24	Silt loam, Acid Cisne soil sampled from plot 309B, Brownstown Agronomy Research Farm Chemical Composition 0.95 % Org-C 1.09 % Fe 406 Mn ($\mu g \cdot g^{-1}$)
B1	Soil sample, Cecil series taken from the P-1 watershed on the Southern Piedmont Conservation Research Center farm in Oconee County near Watkinsville, Georgia Chemical Composition 0.90 % Org-C 1.28 % Fe 990 Mn ($\mu g \cdot g^{-1}$)

removed and analyzed for hydrogen peroxide. Decay studies were all carried out at least in triplicate (in several cases more than triplicate decay rates were determined), the rates were averaged, and the standard deviation of the rates determined. Each kinetic run consisted of five to eight H_2O_2 measurements during the course of the experiment. The individual measurements of H_2O_2 were not duplicated.

Kinetic studies: particle size effects

Several kinetic studies were conducted after removing the larger particles by filtration (0.45 μm filters) or by centrifugation. In addition, some studies were conducted using suspensions that were centrifuged for 2 h at 17 000 G (15 000 rpm). The centrifugation, at 25°C, was sufficient to eliminate all particles greater than 0.02 μm size according to the Stokes Law (Jackson 1956).

Kinetic studies with sterilized soils

Formaldehyde (0.025 M) was added to a suspension of soils 7 and 10 and the treated suspensions were incubated for 15 h at 25°C prior to the addition of H_2O_2. The decay of H_2O_2 was completely stopped (< 5%) for at least 6 h in both suspensions. Controls in buffered distilled water (pH 7 with 0.01 M phosphate), or in buffered distilled water to which 0.025 M formaldehyde had been added, showed no decrease in H_2O_2 concentration over 7.5 h.

The addition of formaldehyde to the soil suspensions resulted in a positive interference to the peroxide assay. That is, the rapid initial decrease in the scopoletin fluorescence, which corresponded to the H_2O_2 concentration, was followed by an additional gradual decrease to a constant value. The total fluorescence change was in every case higher than that with no formaldehyde added. Corrections were made for this interference using calibration curves.

A suspension of soil 12 and a water sample from the Beaver Dam Lake were treated with 0.50 mM $HgCl_2$ (21.7 $mg \cdot L^{-1}$), then incubated for 15 h. H_2O_2 was added to all samples and its decay was followed with time. No decay in H_2O_2 concentration was observed over 7.5 h in the distilled water with or without the addition of Hg(II). The suspension of soil 12, to which Hg(II) had been added, showed no change in H_2O_2 concentration over 8.5 h. The decay of hydrogen peroxide also was followed in suspensions of soil 12 and in Beaver Dam Lake water that contained 0.010 M NaN_3 or that were heat sterilized by boiling for 10 min or by triple autoclaving. The decay was also followed in triply autoclaved water from the Bar-H Lake. It is worth noting that the boiling or autoclaving of both lake water samples resulted in production of H_2O_2 in the 0.1 to 0.2 micromolar range.

Kinetic effects of seeding

Two other triply autoclaved Bar-H water samples were studied in more detail. One of the samples was seeded with 0.3% by volume of the unautoclaved water while the second was maintained under aseptic conditions. An untreated Bar-H sample and both treated samples were incubated in the dark on a shaker table for 7 d at 22°C. These samples were removed from the incubator and were buffered at pH 7 with 0.01 M phosphate. Hydrogen peroxide was added and its decay with time recorded.

Concentration dependence

To determine whether the decay of H_2O_2 was first-order with respect to H_2O_2, different concentrations of H_2O_2 were added to a Bar-H water sample. Three concentrations of H_2O_2 were used, 10, 1.0, and 0.10 μM. The concentration of H_2O_2, in the sample to which 10 μM H_2O_2 had been added, was 0.5 μM after approximately 24 h. This provided a fourth sample with an equivalent initial concentration of 0.5 μM.

TABLE 2. Dark decay, at 25°C, of hydrogen peroxide in soil suspensions. Unless noted the H_2O_2 was added at a concentration of 0.5 μM.

Soil	Rate constant (k_d) $(h)^{-1}$	$t_{1/2}$ (h)	Soil concentration (C_s) $(mg \cdot L^{-1})$	$\times 10^3\ k_d/C_s$ $(L \cdot mg^{-1} \cdot h^{-1})$
7	0.333 ± 0.039[a]	2.08	109	3.2
	$<8 \times 10^{-3}$	>80[b]		
10	0.310 ± 0.014	2.24	91.3	3.4
	$<8 \times 10^{-3}$	>80[b]		
12	0.179 ± 0.021	3.87	46[c]	33.9
	0.165 ± 0.022	4.21		
	$<6 \times 10^{-3}$	>110[d]		
	0.0577 ± 0.0038	12.0[e]		
24	0.129 ± 0.006	5.39	48	2.7
	0.0181 ± 0.0134	38.3[f]		
B1	0.125 ± 0.002	5.55	107	1.2
	0.110 ± 0.004	6.30[f]		
	0.0337 ± 0.004	20.6[g]		

[a]One standard deviation.
[b]0.025 M formaldehyde added to soil suspension.
[c]Estimated concentration based on (*25*).
[d]0.5 mM Hg(II) added to soil suspension.
[e]0.010 M NaN_3 added to soil suspension.
[f]Soil suspension passed through 0.45-μm filter.
[g]Soil suspension centrifuged for 2 h.

Oxidation of p*-anisidine*

p-Anisidine was selected for this study because it is known to be very susceptible to peroxidase-catalyzed oxidation by H_2O_2 (Migler and DeChatelet 1978; Saunders et al. 1964; Daniels and Saunders 1951). *p*-Anisidine (1.00×10^{-4} M) was added to water samples from the Middle Oconee River and the Beaver Dam, to a suspension of the EPA-7 soil, to aqueous horseradish peroxidase (40 mg/L), and to distilled water. The solutions were buffered at pH 7.0 by phosphate (0.01 M). Each solution was split in half and hydrogen peroxide (20 μM) was added to one of the halves. The solutions were placed in a thermostatted bath at 25.0°C. Aliquots were withdrawn after 3.0 and 24 h and immediately filtered (0.45-μm filter). The filtrates were analyzed by high performance liquid chromatography. The major product formed in the systems with added hydrogen peroxide was collected by preparative high performance liquid chromatography and its uv/visible spectrum (λ_{max} 490, 310, 260 nm) and mass spectrum (m/z molecular ion, base 213, 198, 182, 170) were obtained.

Results and Discussion

Hydrogen Peroxide Decay in Soil Suspensions

To study the dark decay of H_2O_2, both soil suspensions and natural water samples were used. Initial studies were conducted on suspensions of five well characterized soils (Table 2). The decay of peroxide, at an initial peroxide concentration of 0.5 μM, was observed to be cleanly exponential out to at least two half-lives. First order rate constants in the samples varied from 0.12 to 0.33 h^{-1}, which correspond to half-lives ($t_{1/2}$) ranging from 5.6 to 2.1 h. In all cases, the decay of hydrogen peroxide was at least an order of magnitude faster in the

sediment suspensions than in distilled water, demonstrating the important catalytic role played by the soils. To compare the catalytic effect of the soils per unit mass, first-order decay rate constants were normalized to the soil concentration ($mg \cdot L^{-1}$) (see k_d/C_s in Table 2). The normalized rate constants generally clustered in a range that varied by less than a factor of four from the lowest to the highest value. Excluding soil B1, which was an outlier, the difference was less than 50% from the lowest to the highest value and the decay rate could be described by equation (1):

$$(1) \quad -d[H_2O_2]/dt = [(3.3 \pm 0.5) \times 10^{-3}]\, C_s\, [H_2O_2].$$

The rate constants did not correlate with either the Fe or Mn content of the soils (Table 1).

Particle size effects

To better define the role of the particles in the decay of peroxide, several experiments were conducted with two of the soils. Peroxide decay kinetics were examined in filtrates (0.45-μm filter) of the suspensions of soils B1 and 24 and in duplicate centrifugates of the B1 suspension. The centrifugation removed particles with diameters down to 0.02 μm. Sharply contrasting results were obtained in these experiments (Table 2). First, filtration on the one hand had little effect on the decay half-life in the suspension of soil B1, increasing it from 5.6 to 6.3 h. Second, filtration of sediment 24, on the other hand, sharply increased the decay half-life from 5.4 to 38 h. Third, centrifugation of B1 greatly increased the decay half-life to 20.6 h, nearly four-fold longer than in the suspension with no centrifugation ($t_{1/2}$ = 5.6 h). These experiments indicated that decay in the suspension of soil 24 was mainly attributable to particles with diameters in the 0.4 to 2 μm range whereas much smaller particles (0.02 to 0.4 μm) were mainly responsible for inducing decay in the suspension of sediment B1.

Kinetic effects of sterilization

Additional studies of H_2O_2 decay were conducted using a series of chemicals and procedures known to kill or inhibit microorganisms. Formaldehyde (0.025 M), a well-known chemical sterilant, reduced the decay rate by at least a factor of 40 in both suspensions. Similar kinetic results were obtained using the non-specific bactericide Hg(II) (0.50 mM) (Brock 1978). The decay rate constant in suspensions of soil 12 was decreased by a factor of at least 30 by addition of the mercuric chloride. The addition of 0.010 M NaN_3 to soil 12 (Table 2) decreased the rate of decay of H_2O_2 by approximately three-fold, but did not totally inhibit the processes leading to H_2O_2 decomposition. This result is consistent with other findings that azide does not inhibit all microbial activity. Azide specifically inhibits the activity of peroxidase enzymes (Migler and Dechatelet 1978). Some bacteria and their associated functions are not inhibited by azide and thus are still capable of destroying H_2O_2.

Kinetic Studies in Turbid Natural Waters

Kinetic studies were extended to natural waters obtained from two turbid impoundments, Bar-H and Beaver Dam. The results indicated that the half-life of H_2O_2 was much shorter in the Bar-H sample, $t_{1/2}$ = 1.0 h, than in the Beaver Dam sample, $t_{1/2}$ = 5.3 h (Table 3). A series of experiments were conducted to elucidate the role of microbiota in the decay of peroxide in the water.

Effects of sterilization and seeding

Chemical sterilization using Hg(II) or azide, and heat sterilization by boiling, resulted in at least an order of magnitude increase in the half-life for hydrogen peroxide in water from the Beaver Dam Lake (Table 3). Likewise, the triple autoclaving of Bar-H Lake water greatly increased the half-life for H_2O_2 decay (Table 3).

Two other autoclaved Bar-H water samples were studied in more detail. One of the samples was seeded with untreated water from the Bar-H Lake and allowed to incubate for a week. The decay rate of H_2O_2 in this sample was the same as that in the untreated Bar-H sample (Table 3). The other autoclaved sample was not seeded but was incubated aseptically over a period of 1 wk. No hydrogen peroxide decay occurred in this sample over a 12-h period. These results further indicate that microorganisms are mainly responsible for catalyzing the decay of H_2O_2 in this natural water.

Concentration dependence

The results of the concentration dependent decay for Bar-H, 12 d after collection, are shown in Table 3. Although there was some scatter, the three lower concentrations resulted in log linear decay with $t_{1/2}$ of 2.1 to 2.7 h. From these results, it appears that the decay reactions are first-order with respect to H_2O_2 at low concentrations. Peroxide decay was slowest in the sample having the highest initial peroxide concentration (50% decay in 8.1 h) and was not log linear. These results are not surprising, given the evidence presented above for the biological mediation of such processes and the possible inhibiting effect of peroxide.

Peroxidase Activity

To learn more about the mechanisms of the decay of hydrogen peroxide catalyzed by sediments, we examined the oxidation of the aniline, *p*-anisidine, by hydrogen peroxide in two natural water samples and in a soil suspension. Results of experiments in the water samples obtained from the Middle Oconee River and Beaver Dam Lake and in the suspension of EPA-7 soil showed that *p*-anisidine was oxidized by hydrogen peroxide to the same major product that formed in buffered (distilled water) peroxidase solution. In the absence of a catalyst, no oxidation of *p*-anisidine by the hydrogen peroxide occurred in buffered distilled water. In peroxidase solutions in buffered distilled water, 1.0 mole of *p*-anisidine was oxidized per mole of added hydrogen peroxide. In comparison, this ratio was found to be 0.55 in the Middle Oconee River water, 0.70 in the Beaver Dam Lake water, and 0.40 in the suspension of EPA-7 soils.

Some effort was made to tentatively identify the major oxidation product. The mass spectrum of this product was consistent with an assigned structure of benzoquinone-4-methoxyanil. This product is similar in structure to the colored product formed in the oxidation of 4-methoxy-2,6-dimethylaniline by hydrogen peroxide and peroxidase at pH 4.5 (Saunders and Watson 1950; Daniels and Saunders 1951). Additional details concerning this reaction will be reported elsewhere.

Hydrogen peroxide and iron(II) also can oxidize substrates via the Fenton reaction (Walling 1975). In this study, however, it was found that the addition of Fe(II) (20 μM) to an aqueous buffered solution (pH 7.0) of 100 μM *p*-anisidine and 100 μM hydrogen peroxide resulted in no detectable formation of benzoquinone-4-methoxyanil. Rapid oxidation to other colored

TABLE 3. Dark decay, at 25°C, of hydrogen peroxide in two natural waters. Unless noted the H_2O_2 was added at a concentration of 0.5 μM. Conc. = concentration.

Water body	Treatment	Decay rate constant, (h^{-1})	$t_{1/2}$(h)
Beaver Dam Lake (1 d after collection)	None	0.13 ± 0.063	5.3
	0.50 mM Hg (II)	$<8 \times 10^{-3}$	>80
	0.010 M NaN_3	$<8 \times 10^{-3}$	>80
	Boiling for 10 min	$<8 \times 10^{-3}$	>80
Beaver Dam Lake (5 d after collection)	None	0.087 ± 0.026	8.0
Bar-H Lake (3 d after collection)	None	0.66 ± 0.065	1.1
	Autoclaved three times	$<8 \times 10^{-3}$	>80
Bar-H Lake (9 d after collection)	None	0.30 ± 0.01	2.3
	Autoclaved then seeded and incubated for 1 wk	0.32 ± 0.002	2.2
	Autoclaved then incubated for 1 wk	$<8 \times 10^{-3}$	>80
Bar-H Lake (12 d after collection)	None, initial H_2O_2 conc. was 10 μM	Decay kinetics not first order	~8.1 (50% decay)
	None, initial H_2O_2 conc. was 1 μM	0.25 ± 0.014	2.7
	None, initial H_2O_2 conc. was 0.5 μM	0.34 ± 0.03	2.1
	None, initial H_2O_2 conc. was 0.1 μM	0.31 ± 0.03	2.2

products did occur, presumably via oxidations involving hydroxyl radicals (Walling 1975).

These product studies suggest that soil-associated peroxidases may be involved in the catalyzed decomposition of hydrogen peroxide in the freshwater studied here. This conclusion is consistent with the above discussed kinetic results concerning inhibition of peroxide decay by azide.

Conclusions

Results of this study indicate that biologically mediated processes were largely responsible for the decay of H_2O_2 in several freshwater samples and soil suspensions. These microbial decay processes, acting in conjunction with the photochemical formation of the peroxide, are likely to be mainly responsible for the observed diel changes in H_2O_2 observed in other fresh water environments. This decay is presumably associated with cellular catalases and peroxidases. In two natural water samples and a soil suspension, *p*-anisidine was oxidized (in the dark) by the addition of H_2O_2 to form a product characteristic of a peroxidase mediated reaction. Additional studies of natural waters are required to determine whether this phenomenon is more broadly applicable. It also is necessary to initiate studies using defined bacterial cultures and to extend studies already reported on algal mediated H_2O_2 decay processes.

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Use of Statistical Models for the Estimation of Abundance from Groundfish Trawl Survey Data

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Estimates of fish abundance from stratified random trawl surveys are highly variable and a number of estimators from various statistical models have been suggested to provide more precise estimates. However, model-based estimates of the survey finite population mean which are not based on the sample mean, can be biased and nonrobust to deviations from the model. This is demonstrated in particular for estimates based on the Δ-distribution. A criterion known as asymptotic design consistency (ADC) is presented for selecting those models that can provide estimates of the finite population mean which are asymptotically robust to deviations from the model. The concept of a predictive estimate is presented as a means of incorporating models into an estimate of the finite population mean which can provide more information than the sample mean. Predictive estimates use statistical models to relate the abundance measured in the sample to covariates measured over the whole survey area. This paper demonstrates that consistent relationships exist between the catch of age 4 cod (*Gadus morhua*) in the survey trawl and concurrently measured hydrographic covariates which can be used to construct model-based ADC predictive estimates of the finite population mean.

Les estimations de l'abondance des poissons obtenues à partir d'enquêtes stratifiées aléatoires de chaluts sont extrêmement variables et un certain nombre de variables d'estimation, provenant de divers modèles statistiques, ont été proposées pour obtenir des estimations plus précises. Cependant, les estimations fondées sur des modèles de la moyenne de la population finie d'un levé, qui ne sont pas fondés sur la moyenne de l'échantillon, peuvent présenter un biais et être non robustes par rapport aux modèles. Cela est démontré notamment pour les estimations fondées sur la distribution Δ. Un critère dit régularité du design asymptotique (ADC) est présenté et permet de choisir les modèles qui peuvent fournir des estimations de la moyenne de la population finie, qui sont robustes de façon asymptotique aux déviations du modèle. Le concept des estimations de prédictions est proposé comme un moyen d'incorporer des modèles en estimations de la moyenne de la population finie qui peuvent fournir plus de renseignements que ne le peut la moyenne de l'échantillon. Les estimations de prédictions, relient l'abondance mesurée dans l'échantillon, à l'aide de modèles statistiques, à des covariables mesurées sur toute la zone d'enquêtes. Le présent article démontre qu'il existe un rapport conforme entre les captures de morues (*Gadus morhua*) d'âge 4 dans les enquêtes de chaluts et les covariables hydrographiques mesurées en même temps qui peuvent être utilisés pour construire des estimations de prédictions (ADC fondées sur un modèle) de la moyenne de la population finie.

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Estimates of abundance from bottom trawl surveys are an important source of information for fishery management on the Atlantic coast of Canada and the United States (Doubleday 1981). Details for specific regions have been given by Pitt et al. (1981); Halliday and Koeller (1981); and Azarovitz (1981). All of the current surveys in these areas use a stratified random scheme, with depth as the major stratifying variable.

The analyses of data from these surveys have been, in general, confined to the estimation of means or total abundance and associated variances using the standard formulae for stratified random designs (e.g. Cochran 1977). The estimates of mean or total abundance are commonly used in the stock assessment process (Doubleday 1981; Anon. 1987a,b). Recently, methods have been developed which either explicitly incorporate the variances of these estimates directly into models of trends in fish abundance over time (Collie and Sissenwine 1983; Gavaris 1988), or can be modified to include these variances (Fournier and Archibald 1982; Deriso et al. 1985). These variances are used to weight associated abundance estimates. In general, abundance estimates from trawl surveys characteristically have large variance estimates, and may change over time faster than the stock dynamics allow (Smith 1988a). Often, the mean and variance are directly related, that is larger means have larger variances associated with them. Therefore, estimates indicating low abundance would be given higher weight than those indicating higher abundance if the variances are used as weights. As a result all but the most severe changes in abundance could be masked by the variation associated with the high abundance estimates. Low precision is not a desirable property for abundance estimates which are used to make public policy decisions. A number of statistical models have been suggested for the estimation of mean catch per tow from any one survey in an attempt to account for this variability. Most of these models are simply skewed probability distributions which were chosen to reflect observed mean to variance relationships, for example; the negative binomial (Taylor 1953; Houser and Dunn 1967), the poisson (Brodie and Wells 1985), and the Δ-distribution (Pennington 1983, 1986; Smith 1988b). This approach

also includes the use of the proposed probability model to define a transformation of the data (e.g. log, square root, etc.) to meet the assumptions of the normal distribution (e.g. Pennington and Grosslein 1978). The usual advantages given for such models include: (a) the provision of more precise estimates of the mean (e.g. Δ-distribution) and (b) the possibility (but not necessarily the means) of defining confidence intervals restricted to positive values.

Although the modelling approach may be seen as simply using an appropriate model to derive estimates for the process being studied, it actually represents a change in the basis of inference from that defined by sample survey theory or design-based theory to a model-based one. For design-based theory, all inferences about the estimates of design-based population quantities are based on treating the measured characteristics (e.g. numbers of fish, etc.) as fixed (Särndal 1978). It is the method of choosing the sample units (e.g. area swept by trawl gear) from a finite population of such units as specified by the survey design which provides the source of randomness. In model-based inference (e.g. distribution fitting, regression analysis, Anova, etc.) the data are assumed to be generated by some underlying stochastic process whose structure is entirely known, apart from a fixed number of parameters (Hannan 1987). The process of sampling only enters the picture through the use of random sampling to ensure that all observations are independent and identically distributed. Therefore the application of models to survey data to estimate design-based quantities such as the finite population mean involves the mixing of two very different philosophies of statistical inference. Little (1983a) notes that for the case of simple random sampling and a normal model the two approaches will lead to similar point and interval estimates for the finite population mean. The two methods can, however, give very different results when other sample designs, especially unequal probability sample designs and/or other models, are used. There are two possible impacts of using models to define estimators for the finite population mean. In the first place the expected value of a random variable for these statistical models may not be equivalent to the finite population mean (e.g. Δ-distribution). Biased estimates could result from using estimators designed for these expected values instead of the finite population mean. In addition, models are always wrong in some sense and the robustness of model-based estimates of the finite population mean to deviations from the model would need to be evaluated.

The purpose of this paper is to investigate the consequences of using statistical models for the estimation of the mean of a finite population. Design-based theory and model-based theory are briefly reviewed with the focus on the differences in their bases of inference. Criteria for evaluating the properties of model-based estimates of the finite population mean are discussed. Using the Δ-distribution as an example of a model for survey data, I show that model-based estimates may not retain their model-based properties in a finite population situation. Estimates of the finite population mean based on the Δ-distribution have asymptotic properties that are not robust to deviations from the model. Models where the sample mean is the model-based estimate do provide robust estimates of the finite population mean. In these cases, however there appears to be little advantage in using the model-based approach over the design-based approach.

Model-based predictive estimates for the finite population mean are discussed as an alternative method of incorporating models into the estimation of survey quantities. These estimates use relationships between the variable measured in the sample (e.g. abundance) and covariates which have been measured for the whole sample area to predict the unobserved values of the variable over unsampled areas. I suggest that model-based predictive estimates of abundance which meet the criterion discussed in this paper can be more informative than the sample mean and are useful for a wider range of situations than the analogous design-based ratio and regression estimates. Poisson log-linear models relating the abundance of cod (*Gadus morhua*) at a particular age to hydrographic variables are presented for two bottom trawl surveys of the Scotian Shelf in 1986 and 1987. The results of these analyses demonstrate that there are strong and consistent relationships between the observed catch per tow of age 4 cod over the 2 yr and the concurrently measured hydrographic variables, which can be exploited for predictive type estimation.

Design-based Theory

The basic ingredients of a probability sampling design are: (a) a finite population of unique and identifiable sample units (e.g. location swept by trawl gear); (b) for each unit of this population, a characteristic or a vector of characteristics (numbers, weights, size composition, etc.) which is observed without error; and (c) a sampling plan which assigns a known probability of selection to the sample units (Basu 1971; Smith 1976; Särndal 1978). For the case of a simple random sample (SRS) without replacement, we will consider a finite population of N sample units with a characteristic y_i to be observed where $i = 1,\ldots,N$. An indicator variable I_i will be used to denote whether the ith sample unit is in the sample, i.e.

$$I_i = \begin{cases} 1, \text{ if unit } i \text{ is in the sample} \\ 0, \text{ otherwise.} \end{cases}$$

A sample of $n < N$ units is randomly chosen such that $I_i = 1$ and y_i $(i = 1,\ldots,n)$ are observed for some appropriate rearrangement of the indices. The probability of any sample of size n being selected is $\binom{N}{n}^{-1}$. Note that this probability does not include y_i and therefore is independent of the observed characteristic. The y_i are assumed to be fixed so that it is theoretically possible to observe all N sample units and therefore know everything about the characteristic being measured.

In design-based theory all inferences are based on the above probability for the SRS based designs. Estimators of population values such as the population mean, $\bar{Y}$ are usually chosen to be unbiased over all possible samples. This definition of unbiasedness is often denoted as design-unbiasedness to differentiate it from model-unbiasedness. The model-based property will be discussed in the next section. We denote the expectation operator for finite populations here as $E_D[T]$, for some estimator T. This expectation is evaluated as

$$(1) \quad E_D[T] = \frac{\sum_{k=1}^{\binom{N}{n}} T_k}{\binom{N}{n}} .$$

For a SRS design, $E_D[\bar{y}] = \bar{Y}$, that is, the sample mean is a design-unbiased estimate of the finite population mean. This form of unbiasedness holds without qualification for any finite population of y_i and for any sample size n. The variance of the mean due to observing a sample from the population is

$$\text{Var}(\bar{y}) = E_D[(\bar{y}-\bar{Y})^2]$$
$$= \frac{(N-n)S^2}{Nn},$$

where

$$S^2 = \frac{\Sigma_{i=1}^{N}(y_i-\bar{Y})^2}{N-1}.$$

The next property to be introduced is that of design consistency. An estimate is design consistent if it is equivalent to the population value it is estimating when $n = N$. Consistent estimators may also be biased; for example, the design-based ratio and regression estimators (Cochran 1977).

The precision of any estimate such as the sample mean depends directly upon the form of the estimator and the sampling plan. Within the context of design-based theory, methods of increasing the precision of an estimator always involve the sampling plan. A common example used to illustrate this concept is the comparison of the variance of the mean from a stratified random design (STR) with that from a SRS design. One sample design is said to give a more efficient estimate than another if its associated variance is smaller for the same total sample size. The variance from the STR will be smaller than that from a comparable SRS if samples are allocated to strata in proportion to the size either of the strata or of the variance in the strata. Again, no distributional assumption for the y_i is required for this result. However, the optimal sample size to strata allocation plan does require some a priori knowledge of the expected variance of the mean in each stratum.

Confidence intervals for the sample mean in the SRS case are based upon the expected distribution of $\bar{y}$ obtained from repeated sampling of the finite population. The normal distribution is usually chosen as the limiting distribution for the mean with this choice based on applying the Central Limit Theorem to sampling from a finite population (Hájek 1960). Although this result will hold for any population of y_i with a finite standard deviation, how large n must be before these results are useful is affected by the distribution of the y_i values in the population being sampled (Cochran 1977; Little and Rubin 1987).

Finally, the notation for STR designs is defined here because I will be referring to these designs later on in the paper. All of the preceding discussion applies because sampling is SRS within each stratum. The total number of sample units within each of the L strata and the number sampled are denoted by N_h and n_h ($h=1,\ldots,L$), respectively, with $N = \Sigma N_h$ and $n = \Sigma n_h$. The population and sample mean are given as

$$\bar{Y} = \sum_{h=1}^{L} \frac{N_h}{N}\bar{Y}_h$$

$$\bar{y} = \sum_{h=1}^{L} \frac{N_h}{N}\bar{y}_h,$$

where the index h refers to the stratum. The variance of the sample mean is estimated by

$$\text{Var}(\bar{y}) = \sum_{h=1}^{L} \frac{N_h(N_h-n_h)}{N^2}\frac{s_h^2}{n_h},$$

where $s_h^2 = \Sigma_{i=1}^{n_h}(y_{hi}-\bar{y}_h)^2/(n_h-1)$.

Use of Models for Survey Data

Models For Means and Variances

Whereas in design-based theory inferences are based on averaging over all possible samples, in model-based theory one conditions on the achieved sample (Smith 1983; Valliant 1987). That is, model-based estimators of the mean and their respective variances are derived with respect to the n observations and the model being used. This is in contrast to design-based theory where estimators are derived with respect to the n observations and the survey design which specifies how any n observations are to be chosen. The properties of unbiasedness, precision, consistency, etc. of a model-based estimate are specific to the model. I will illustrate the effect this difference in basis can have by using the Δ-distribution as an example of a model that might be used for survey data.

The Δ-distribution is a log-normal distribution modified to include observations of zeroes. Pennington (1983, 1986) suggested that this distribution may be a useful model for the number of fish (or plankton) caught in net surveys. MacLennan and MacKenzie (1988) have used the Δ model for the abundance of fish estimated from acoustic surveys.

Let $y_1,\ldots,y_n$ be a random sample of size n from a population where the proportion of zero values is represented by δ. For those y_i not equal to zero, the distribution of $z_i = \ln y_i$ is normal with mean μ and variance σ^2. The model-based expectation of y_i, $E_M[y_i]$ ($i=1,\ldots,n$) is given as

$$E_M[y_i] = (1-\delta)\exp\left(\mu + \frac{\sigma^2}{2}\right)$$
$$= \kappa.$$

Note that this result applies for all i and as such defines the mean for the distribution function only. While the model-based expectation of any y_i will provide information on any other y_i, the design-based expection for any one y_i does not have any meaning for any other y_i.

Although $\bar{y}$, the sample mean, is an unbiased estimate of κ, there are other unbiased estimates which have smaller variances. Let n_1 represent the number of nonzero values observed in the sample. Further, let $\bar{z}$ be the sample mean and s^2 the sample variance of the z_i. The minimum variance unbiased estimate of κ is (Aitcheson and Brown 1957),

$$\hat{\kappa} = \begin{cases} \frac{n_1}{n} e^{\bar{z}} g_{n_1}\left(\frac{1}{2}s^2\right) & n_1 > 1 \\ \frac{y_1}{n} & n_1 = 1. \\ 0 & n_1 = 0 \end{cases}$$

The function $g_{n_1}(\,)$ belongs to the class of generalized hypergeometic functions. See Smith (1988b) for further details on the properties of this function and related functions.

The fact that $\hat{\kappa}$ is more precise than $\bar{y}$, i.e. $E_M(\hat{\kappa}-\kappa)^2 \leq E_M(\bar{y}-\kappa)^2$, when the Δ model is correct, has been given as a statistical advantage for using this model for trawl survey data (Pennington 1983; Smith 1988b). The important question here is whether or not $\hat{\kappa}$ retains its model-based properties in a design-based situation.

The following framework is commonly used to investigate the behaviour of model-based estimates in design-based situations. I will confine discussion to a simple random sample design for simplicity. The following ideas can be extended to a stratified random design by the addition of the subscript h used in the previous section. Define the set of observations from a finite population, $y_1,\ldots,y_N$, to be a random sample of size N from an infinite population which has a Δ-distribution with parameters δ, μ, σ^2. The infinite population is often referred

to as the superpopulation in the sampling literature (e.g. Rao 1975). Assume that we observe a random subsample of size n from the N elements which will be denoted as $y_1,\ldots,y_n$ for some suitable rearrangement of indices.

When κ is the target parameter the properties of the estimates are evaluated according to the model. As a result, all of the model-based properties given above for $\hat{\kappa}$ relative to $\bar{y}$ when both are estimated from the subsample of size n will continue to apply in the superpopulation framework. On the other hand, the objective of a trawl survey is the estimation of $\bar{Y}$ and the properties of the estimates have to be evaluated according to this objective. That is, the expectation of $\hat{\kappa}$ would be evaluated according to (1) and unbiasedness would imply that $E_D[\hat{\kappa}] = \bar{Y}$. The higher precision of $\hat{\kappa}$ relative to $\bar{y}$ would be evaluated by comparing the finite population mean squared errors and showing that $E_D(\hat{\kappa}-\bar{Y})^2 \leq E_D(\bar{y}-\bar{Y})^2$.

However, there is no way of showing either of the above conditions hold because $\bar{Y}$ and κ are not the same thing for any one realization of N observations from the superpopulation. There is no basis in statistical theory to enable one to predict the relationship between the average value of $\hat{\kappa}$, taken over all possible subsamples of size n from N, with that of $\bar{Y}$. The formal proof that a uniformly minimum variance unbiased estimator of $\bar{Y}$ does not exist was given by Godambe (1955; see also Basu 1971). The only property that we can evaluate fully here for the finite population is design-based consistency which was defined in the previous section. It is easy to show that the Δ mean is not a design-consistent estimator of the finite population mean because $\hat{\kappa} \neq \bar{Y}$ as $n \to N$.

Another way of contrasting the two bases of inference is that model-based estimates are used to make inferences about parameters of the model, while sample surveys are directed toward inferences about that portion of the finite population which did not appear in the sample. That is, denote the n sample units which are included in the sample as indexed by $i \in s$ and the remaining $N - n$ units not in the sample by $i \notin$ s. The finite population mean can be written as

$$\bar{Y} = N^{-1}\left(\sum_{i\in s}^{n} y_i + \sum_{i\notin s}^{N-n} y_i\right)$$

(2)

$$= N^{-1}\left(n\bar{y} + \sum_{i\notin s}^{N-n} y_i\right)$$

Given that we have observed $\bar{y}$ the problem becomes one of predicting the values of the y_i in the unobserved portion of the population. In a survey design context the sample mean from the observed portion is essentially used to do just that. A model derived from the observed sample, such as the Δ, can also be used to predict the mean of the unobserved portion such that we have,

$$(3)\quad \hat{\bar{Y}}_{\hat{\kappa}} = N^{-1}(n\bar{y} + (N-n)\hat{\kappa}).$$

Within the context of design-based theory we still have the problem that there is no uniformly minimum variance unbiased estimate for $\bar{Y}$ even if we use the predictive estimate in equation (3). However, the predictive estimate will be a design consistent estimate of $\bar{Y}$.

In the spirit of using a model, we could ask how well model-based estimates would perform on average over repeated realizations from the superpopulation. A simple Monte-Carlo experiment was constructed to look at the problem from this viewpoint. Observations from a Δ distribution were simulated by taking the product of a series of pairs of pseudorandom numbers. The first number of each pair was obtained from a Bernoulli random number generator with probability of observing a nonzero observation equal to 0.6 while the second came from a log-normal random generator ($\mu = 0$, $\sigma^2 = 4.0$). A total of 100 finite populations of size $N = 30$ were generated and all possible samples of size $n = 3$ were taken from each one. For each population the design-based expectation and mean-squared errors were calculated for each of the estimates, $\bar{y}$, $\hat{\kappa}$ and $\hat{\bar{Y}}_{\hat{\kappa}}$. The frequency distributions of the percent bias, i.e. $100 \times (E_D[\text{estimate}] - \bar{Y})/\bar{Y}$, where estimate corresponds to either the $\hat{\kappa}$ or $\hat{\bar{Y}}_{\hat{\kappa}}$ for each replication, are presented in Figs. 1a and b. The percent bias of $\bar{y}$ was always equal to zero as expected. The frequency distribution of the relative difference between the mean-squared errors of each of the Δ based estimators and the mean-squared error of $\bar{y}$ are presented in Figs. 2a and b. These relative differences were calculated as the difference between the mean-squared errors of the model-based estimate and $\bar{y}$ and then divided by the associated mean-square error for $\bar{y}$.

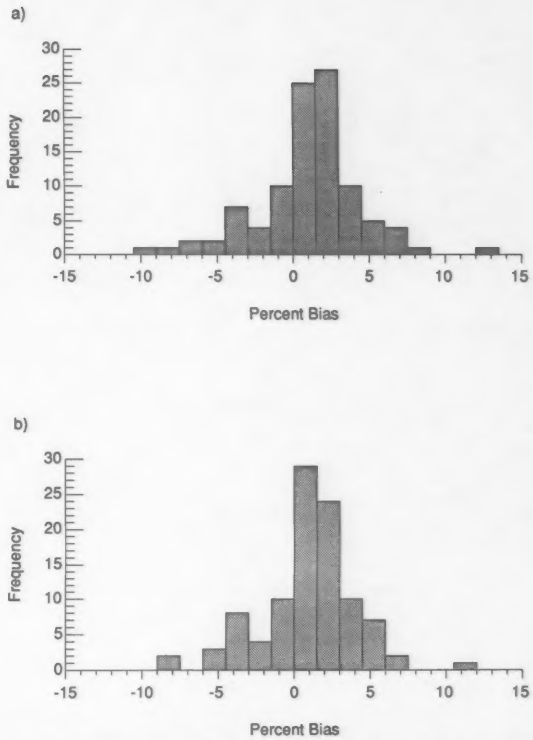

FIG. 1. Frequency distribution of percent bias from Monte-Carlo experiment for (a) $\hat{\kappa}$ and (b) $\hat{\bar{Y}}_{\hat{\kappa}}$.

This simulation was not intended to be comprehensive but it does illustrate a number of important points. For any one realization from the superpopulation the model-based estimates can deviate in expectation from $\bar{Y}$ (Figs. 1a,b). In addition, for the case reported here, both of the model-based estimates had higher variances than the sample mean 81 out of 100 times. These results confirm the point made about the nonexistence of uniformly minimum variance unbiased estimators. When we

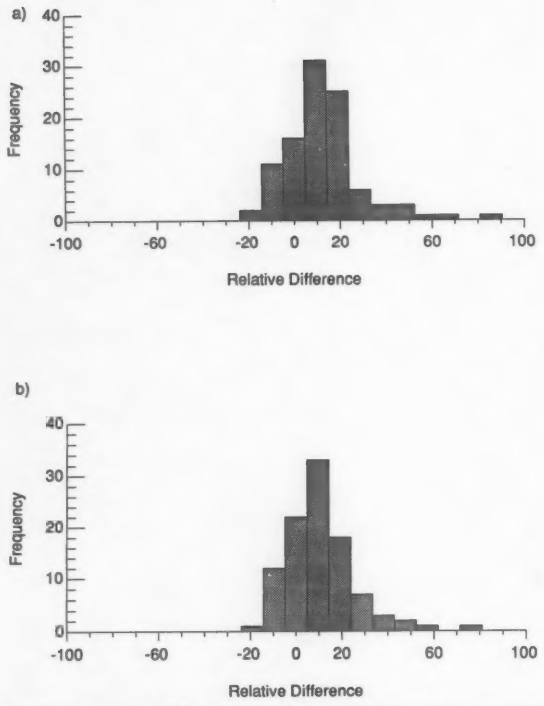

FIG. 2. Frequency distribution of relative difference of mean-squared errors from the Monte-Carlo experiment for (a) $\hat{\kappa}$ and (b) $\hat{\bar{Y}}_{\hat{\kappa}}$.

TABLE 1. Results of the Monte-Carlo study. Entries in table are averages of finite population quantities from 100 replications from a Δ-distribution. Each finite population consisted of 30 populations units from which all possible samples of three sample units were chosen.

Estimator	Percent bias	Mean-squared error	Variance
$\bar{y}$	0.0	433.39	433.39
$\hat{\kappa}$	1.03	405.41	405.17
$\hat{\bar{Y}}_{\hat{\kappa}}$	0.92	406.17	405.99

take the average results over all 100 finite populations we find that the average percent bias for the results given in Fig. 1 was about 1% in both cases (Table 1). This fairly minimum bias may be a function of the number of replications from the superpopulation used in the simulation and as such the bias would be expected to decrease if more replications were employed. The bias may also reflect a certain degree of lack of fit of the simulated data to the Δ model. The results for the mean-squared errors indicated that on the average over the 100 replications the variances for both of the Δ based estimates were smaller than that of the sample mean (Table 1). Therefore the model-based properties of the estimates do not hold for any one realization from the superpopulation, but may hold on the average over many realizations.

The concept of asymptotic design-consistency (ADC) (Brewer 1979; Little 1983a) extends this type of investigation unencumbered by the limitations of simulation studies, to any distribution for any value of N and n. Briefly, this is evaluated by generating t replicates of a finite population such that n/N is constant over all replications for a specific sampling scheme. The t populations and samples are aggregated to form one population and one sample. Then, the behaviour of the estimate is determined as t is increased to infinity. Formally, this is defined as (Wright 1983),

$$(4) \quad \lim_{t\to\infty} (\bar{Y} - E_D[\hat{\mu}]) = 0,$$

where $\hat{\mu}$ represents any model-based estimate of $\bar{Y}$. The concept of a superpopulation model is the basis for generating t replicates of the finite population. The ADC property has also been called asymptotic design-unbiasedness but because it resembles model-based consistency more than it does unbiasedness (Little 1983a), the former name will be used here. Note that both n and N will increase to infinity such that the sampling ratio is constant. The form of the model being used does not explicitly appear in equation (4) and because of this the property of ADC has been promoted as a necessary but not a sufficient condition for the robustness of a model-based estimate of the finite population mean (Brewer et al. 1988; Tam 1988a,b). Little (1983a,b) has proposed that only ADC estimates of the finite population mean be considered in order to protect against model misspecification. Statistical models are used to mimic some aspect of the system under study and as such are never correct. The ADC property ensures that, asymptotically at least, the expected value of the model-based estimate of the finite population mean is independent of the model being used. This is an important property for estimates of fish abundance which may be used to make public policy decisions on allowable catch levels.

The evaluation of the left hand side of equation (4) for the Δ model (i.e. $\hat{\mu} = \hat{\kappa}$) proceeds as follows. As t goes to infinity $E_D[\hat{\kappa}] \to \hat{\bar{\kappa}}$. So the limit in equation (4) will be $\bar{Y} - \hat{\bar{\kappa}}$. When the Δ model is correct then both $\bar{Y}$ and $\hat{\bar{\kappa}}$ will approach κ and the condition in equation (4) will be met. However, if the Δ model is not correct then the limit will simply be some constant equal to $\bar{Y} - \hat{\bar{\kappa}}$ no matter how large t is. This result applies to both of the Δ-based estimates of $\bar{Y}$. Therefore, a model-based estimate of $\bar{Y}$ based upon the Δ distribution is dependent upon the model and as such is not a robust estimate of the finite population mean. Jolly and Smith (1989) present a heuristic argument for the same basic conclusion.

Probability distributions such as the normal, the poisson, and the negative binomial are examples of statistical models which can be used to construct ADC estimates of the finite population mean. In these cases the model-based estimate is the sample mean and the resulting estimates look no different than their design-based counterparts. Apart from the possibility of constructing confidence intervals or hypothesis tests there appears to be little advantage in using these models over design-based methods. Although the poisson and the negative binomial based estimates may have smaller model-based variances associated with them than the design-based sample mean, it would be difficult to accept this increase in precision as a consequence of simply assuming a model for the data.

Model With Covariates

Predictive estimators, based on the framework in equation (2), have been used to construct model-based estimates which can have the ADC property and provide more information than the sample mean (e.g. Little 1983a). Rewrite equation (2) such that a model-based estimate, $\hat{\mu}_i$ is substituted for each of the y_i where $i \notin s$ to give estimate of the general form,

$$(5)\quad \hat{\bar{Y}} = N^{-1}\left(n\bar{y} + \sum_{i=1}^{N-n} \hat{\mu}_i\right).$$

The definition of individual $\hat{\mu}_i$ in the above equation implies that we have information available on all N units in the finite population which can be used to estimate the y_i for those units not in the sample of n units. This information is usually assumed to be contained in a covariate, x_i or a vector of covariates $\mathbf{x}_i$ which have been measured for all of the sample units and are related to the y_i. This relationship is estimated from the observations of y_i and $\mathbf{x}_i$ in the sample. Predictions from this relationship denoted as $\widehat{\mu(\mathbf{x}_i)}$ are used to estimate the y_i in the population units not in the sample. The statistical model therefore consists of a systematic component reflecting the relationship between the y_i and the covariates and a probability model incorporating the assumed mean to variance relationship of the residuals from the systematic component.

Ratio and regression estimates of the finite population mean (e.g. Cochran 1977, chapters 6 and 7) are the design-based analogues of this type of model-based predictive estimates. Neither of these design-based estimators require an explicit assumption of a probability distribution nor do they require that the relationship between the y_i and the covariate(s) be linear. However, the least-squares basis of the coefficients for the regression estimate does implicitly assume that errors are at least symmetric with a constant additive variance. The ratio estimate is actually a special case of the regression estimate when the variance is proportional to the covariate(s) and the regression of y_i on the covariate(s) passes through the origin. The estimates of the finite population mean from the regression or ratio method are design-biased, albeit design-consistent, when the coefficients for the covariates are estimated from the sample. This bias will increase for increasing nonlinearity of the relationships between the y_i and the covariates. It may be possible to extend the design-based estimators to a nonlinear situation but even for least-squares in the nonlinear case errors are assumed to be constant and additive.

The main point to be made here is that model-based predictive estimates may be applicable to a wider range of situations than the design-based methods. The use of generalized linear models (McCullagh and Nelder 1983) to construct predictive estimates of the finite population mean would allow one to deal with situations where there is nonconstant variance and/or nonlinear relationships. The property of ADC would then be used to screen such models to find those which give robust estimates of the finite population mean (Little 1983a).

In situations where the design-based estimate is applicable, the appropriate ADC model-based predictive estimate will often be of the same general form. To illustrate this, consider the following example from Little (1983a) for a stratified random design where we have L strata ($h=1,\ldots,L$) and samples of size $n_h (i=1,\ldots,n_h)$ within each stratum. Assume that the response variable, y_{hi} is normally distributed with expectation under this model given as $E_m[y_{hi}] = \beta_{0h} + \beta_1 x_{hi}$ for any one arbitrary covariate whose values are known over all i. Substituting this model into equation (5) will give, after some algebraic manipulation,

$$(6)\quad \hat{\bar{Y}}_h = (\bar{y}_h + \hat{\beta}_1(\bar{X}_h - \bar{x}_h)),$$

where $\bar{X}_h$ and $\bar{x}_h$ are respectively, the population and sample mean of the covariate. In this example the least squares estimate of β_1 is pooled over strata and weighted by the stratum weights N_h/N. The estimate in equation (6) is ADC because as $t \to \infty$ the sample mean of x_h will approach the population mean and the term $\hat{\beta}_1(\bar{X}_h - \bar{x}_h)$ will approach zero. This will result in $E_D[\hat{\bar{Y}}] \to \bar{Y}_h$ and the limit in equation (4) will also approach zero. Note that this estimator will be ADC even when the assumption of a common slope across strata is incorrect. Little (1983a) has also pointed out that models that fit separate intercepts for each stratum are always ADC, hence the inclusion of β_{0h} in the model. This estimator is almost identical to the univariate form of the regression estimator from design-based theory. The difference between equation (6) and the regression estimate is that the coefficient $\hat{\beta}_1$ in the former refers to a parameter of the superpopulation while the estimate in the design-based case is a consistent, albeit biased estimate, of the finite population slope B. Cochran (1977, page 199) has shown that $\hat{\beta}_1$ is an unbiased estimate of the superpopulation parameter β_1.

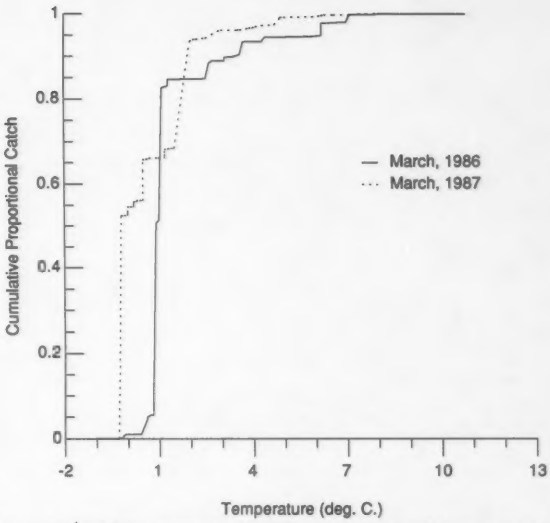

FIG. 3. Cumulative proportional catch of age 4 cod and the associated bottom temperature measured during the March 1986 and 1987 trawl surveys of NAFO Subdivision 4Vs and Division 4W.

The estimator given in equation (6) also illustrates one of the more useful aspects of this kind of estimator when it is ADC. The covariate correction to the sample mean will only be applied if the mean of the covariate in the sample differs from that in the population. That is, the covariate adjustment is in response to conditions being different on the average in the strata from what was observed in the sample. No adjustment is necessary if conditions were the same.

An Application

The application of the predictive estimate given in equation (5) requires the development of useful models for the distribution of some species of fish and a set of covariates that can be measured over a large area. In this section models are presented which relate the number of age 4 cod caught per tow from groundfish trawl surveys in NAFO Subdivision 4Vs and Division 4W, to environmental variables which were measured routinely at each tow site. The environmental variables measured were bottom temperature, salinity, and depth. Scott (1982) has shown that the distribution of cod appears to be related to all of these variables. This investigation was restricted to one

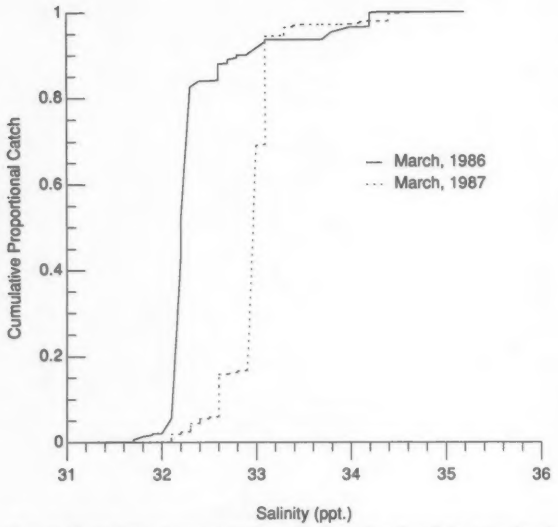

FIG. 4. Cumulative proportional catch of age 4 cod and the associated bottom salinity measured during the March 1986 and 1987 trawl surveys of NAFO Subdivision 4Vs and Division 4W.

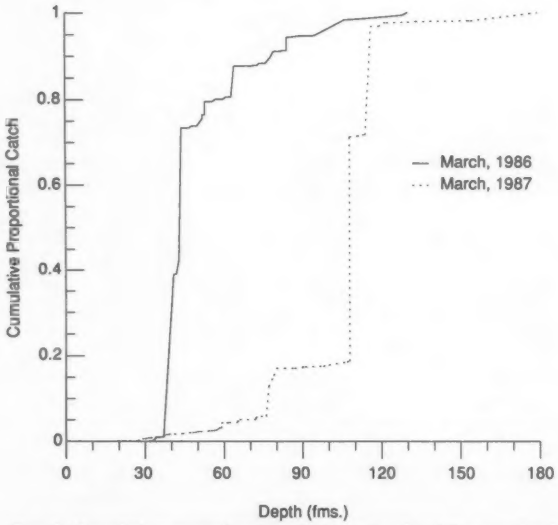

FIG. 5. Cumulative proportional catch of age 4 cod and the associated bottom depth measured during the March 1986 and 1987 trawl surveys of NAFO Subdivison 4Vs and Division 4W.

age because cod demonstrate age-specific relationships to these environmental variables (Tremblay and Sinclair 1985; Sinclair and Smith 1987).

The data from two stratified random surveys (March 1986 and 1987) of the 4VsW cod stock were chosen for this analysis. Details of the general cruise procedures are given in Halliday and Koeller (1981) and in Koeller (1981a). The cumulative catches of 4-yr-olds for each cruise, presented as proportions of the total catch of age 4 cod, are plotted against the observed temperatures, salinities, and depths in Figs. 3–5. The patterns in the figures are more interpretable when the environmental conditions for the 2 yr are compared. The stratified mean temperature (±one standard error) in 1987 was 2.4°C (±0.26), which was cooler than that observed in 1986 (3.4°C (±0.32)). The stratified estimates of the median temperature were 3.69°C in 1986 and 1.94°C in 1987. Despite these differences, the majority of the catches of cod in both years were associated with temperatures of ≤ 2°C. This relatively cooler water was associated with higher salinities and deeper depths in 1987 than in 1986. However, the salinities that most of the cod were associated with in both years (32–33.5 ppt.), correspond to the range identified for the cold intermediate water mass by Hachey (1942).

The pattern for any one of the covariates indicates a range of values over which the probability of cod being present is higher than that for values outside of this range. This would suggest nonlinear relationships between the number of cod caught and any one of the covariates. The probability model was chosen to reflect the observation that the mean and variance of the numbers caught in trawl surveys appear to be positively related (e.g. Taylor 1953; Forest and Minet 1981; Koeller 1981b; Gavaris and Smith 1987). This would suggest that a skewed type of distribution be considered for the probability model. The following model based on a general form of the poisson distribution appears to take these considerations into account. Let y_{hi} represent the number of age 4 cod caught in sample i of stratum h and $\mathbf{x}_{hi}$ represent a vector of p covariates with elements x_{hik}. The model is defined as

$$E_M[y_{hi}] = \mu(\mathbf{x}_{hi}),$$
$$\mathrm{Var}_M[y_{hi}] = \phi\mu(\mathbf{x}_{hi}),$$

where ϕ is a measure of the expected extra-poisson variance. Note that $\phi = 1$ would result in a standard poisson variance. Lawless (1987) discusses poisson regression models in the presence of extra variation. The relationship chosen between the expected value and the covariates was

$$\mu(x_{hik}) = \exp\left\{\beta_{h0} + \sum_{k=1}^{p} \beta_k x_{hik}\right\},$$

which can also be written as

$$\mu(x_{hik}) = \bar{y}_h \exp\left\{\beta_0' + \sum_{k=1}^{p} \beta_k x_{hik}\right\}.$$

The latter form reflects the proportional adjustment of the covariates on the stratum mean, used here as a measure of local abundance. That is, even though conditions may be equally favourable in two separate areas, the number of fish caught in any one set in each area will be assumed to be a proportional function of the local density. The exponential term also ensures that the predicted values will be greater than or equal to zero.

A predictive estimate of the form in equation (5) for the stratified mean based on this poisson model will be an ADC estimate. To see this rewrite equation (5) for the mean of the hth stratum as,

$$(7)\quad \hat{\bar{Y}}_h = N_h^{-1}\left(n_h \bar{y}_h + \sum_{i \notin s}^{N_h - n_h} \mu(\mathbf{x}_{hi})\right),$$

and rearrange terms to get

$$\hat{\bar{y}}_h = N_h^{-1}\left(n_h \bar{y}_h - \sum_{i \in s}^{n_h} \mu(\mathbf{x}_{hi}) + \sum_{i=1}^{N_h} \mu(\mathbf{x}_{hi})\right)$$

The term $(n_h \bar{y}_h - \Sigma_{i \in s}^{n_h} \mu(\mathbf{x}_{hi}))$ will always equal zero for the

TABLE 2. Estimates of model parameters and associated standard errors (SE) for 1986 and 1987 surveys (age 4 cod).

	Survey years			
	1986		1987	
Parameters	Estimates	SE	Estimates	SE
Salinity	87.28	37.28	105.8	26.97
$(\text{Salinity})^2$	−1.294	0.5688	−1.570	0.4083
Temperature	−0.8833	0.3625	−0.6787	0.1479
$(\text{Temperature})^2$	0.0435	0.0457	0.0025	0.0520
Depth	0.1696	0.0627	0.1688	0.0232
$(\text{Depth})^2$	−0.0009	0.0004	−0.0007	0.0001

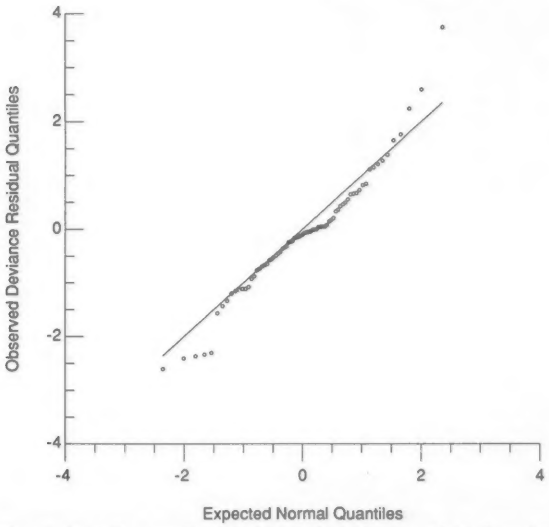

FIG. 6. Quantile–quantile plot of the scale deviance residuals from the poisson model for age 4 cod from the March 1986 cruise.

poisson distribution if a constant term (or intercept) is included in the model (McCullagh and Nelder 1983). As $t \to \infty$ in equation (4) the x_{hik} in the sample will approach their range of values in the population and the difference between the finite stratum population mean and $E_D[\hat{\bar{Y}}_h]$ will approach zero.

The models were fitted to the data using the GLIM software (McCullagh and Nelder 1983; Payne 1986). Estimates of the model parameters and their associated standard errors are presented in Table 2. The estimates of $\hat{\phi}$ for the 1986 and 1987 were 48.77 and 2.951, respectively and these have been incorporated into the standard error estimates. Plots of residuals from the fit of linear terms only in the transformed scale suggested the addition of quadratic terms to the model. These terms were significant for salinity and depth but not so for temperature. The most striking feature of Table 2 is the similarity of the estimated coefficients over the two years. All of the estimates for each set of covariates are within two standard errors of each other for the two years. In particular, the coefficients for the depth terms are almost identical. Although the pairwise correlations between the covariates were significant at $p<0.02$, these correlations were not serious enough to result in aliasing of any of the covariates. However, this collinearity has probably resulted in some loss of precision of the estimates of the coefficients (Greenberg 1975; Gunst and Mason 1977).

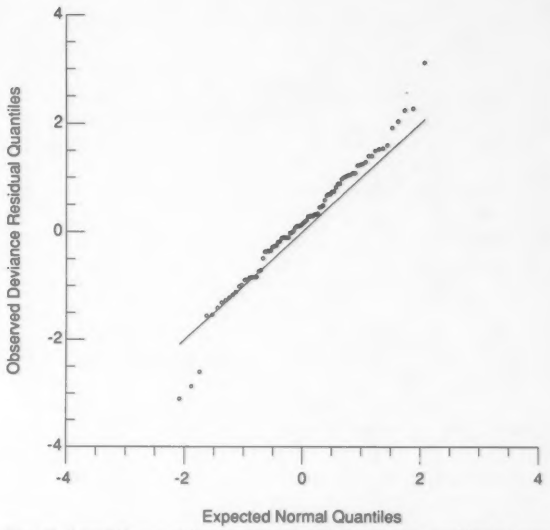

FIG. 7. Quantile–quantile plot of the scaled deviance residuals from the poisson model for age 4 cod from the March 1986 cruise.

The appropriateness of the poisson model for the data was investigated by plotting the quantiles from the scaled deviance residuals (McCullagh and Nelder 1983) against the expected quantiles from a standard normal distribution (Figs. 6 and 7). Deviance residuals have been found to be approximately normally distributed when the model is correct and are therefore useful for goodness-of-fit evaluation (Pierce and Schafer 1986). No gross departures from normality are evident in these figures. In fact, comparison of these plots with those presented in Chambers et al. (1983) for samples generated from a normal pseudo-random generator would indicate that the fit of the poisson model was very good. The stragglers or outliers at either end of the plots correspond to cases where either no fish were caught and the model predicted a catch (negative residuals) or a prediction of no fish when some were observed (positive residuals). In all these cases the maximum number of fish involved whether predicted or observed was 10. Removal of these points from the analysis did not significantly affect the estimates of the coefficients.

These results show that there are definite and consistent relationships between hydrographic conditions and the numbers of age 4 cod caught in the net. Furthermore, the temporal consistency of these estimates would support the use of a model similar to that developed here to predict the abundance of age 4 cod over unsampled areas where only the hydrographic information was available. Finally, the poisson model with its associated ADC properties will provide a robust estimate of the finite population mean. The results presented here appear to be encouraging enough to extend these analyses over more years, areas, age groups, and species.

At present, measurements for these hydrographic covariates are routinely made at trawl sites only and therefore no information is available on conditions over the nonsampled areas at the same time that the survey was carried out. However, as a first step it is important to establish if relationship exist between abundance and these covariates before resources are committed to obtaining environmental information over the whole area. Application of this predictive estimator to these data would not

change the estimates of mean abundance unless hydrographic conditions in the unsampled areas were different, on the average, than what was observed for the trawl samples. For example, if the appropriate temperatures and salinities had occurred in shallower water in the unsampled areas in 1987 more than what was observed, then the estimate of age 4 cod would be increased over what was estimated from the survey. On the other hand, if there were fewer areas with suitable conditions in 1986 than what was observed, the estimate for that survey would be decreased. Either way, these surveys may be indicators only of the abundance of cod near bottom and available to the gear and not of the total abundance of the stock. This may have some relationship to the commercial fishery at the same time and for similar gears, but this aspect needs further investigation.

The variance of the predictive estimate for the mean of the h strata is given in the appendix. This estimate will provide increasingly larger estimates of the variance as the differences between the covariate sample means and means from the unsampled portion increase. Such behaviour reflects the consequences of placing more reliance upon the model to estimate the mean and is typical of these kinds of estimates. Although the estimate of the finite population mean presented in this section is ADC, further work is required to see if this variance estimate is also ADC. Even if this variance estimator is not ADC it is still useful for evaluating the information in the covariates. That is, one could use the magnitude of the difference in the variance, depending upon whether or not covariates are used in the model to predict over the unsampled portion, as a measure of the reliability of the survey.

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Appendix

The variance of the predictive estimate given in equation (7) of the text is obtained as follows.

$$E_M(\hat{\bar{Y}}_h - \bar{Y}_h)^2 = N_h^{-2} E_M\left(\sum_{i \notin s}^{N_h - n_h} \hat{\mu}_h(x_{hi.}) - \sum_{i \notin s}^{N_h - n_h} y_{hi}\right)^2.$$

The right side, after some algebraic manipulation can be expressed as consisting of two variance components, i.e.

$$E_M(\hat{\bar{Y}}_h - \bar{Y}_h)^2 = N_h^{-2}\left\{\mathrm{Var}_M\left(\sum_{i \notin s}^{N_h - n_h} \hat{\mu}_h(x_{hi.})\right) + \mathrm{Var}_M\left(\sum_{i \notin s}^{N_h - n_h} y_{hi}\right)\right\}.$$

These variance components are evaluated by applying the results given in section 4 of Valliant (1985) to the poisson distribution to give the variance of the predictive estimate as,

$$\mathrm{Var}(\hat{\bar{Y}}_h) = N_h^{-2}\left\{\sum_{i \notin s}^{N_h - n_h} \mu_{hi}^2 X_h \mathrm{Var}_s(\beta) X_h' + \sum_{i \notin s}^{N_h - n_h} \mu_{hi}\right\},$$

where X_h is an $(N_h - n_h) \times p$ matrix of covariates from the nonsampled part of the strata and $\mathrm{Var}_s(\hat{\beta})$ is the $p \times p$ matrix of the estimated variances and covariances of the estimates of coefficients based upon the sample. The estimate of the variance of the predictive estimate is obtained by substituting sample estimates for the parameters in the above formula.

Validation of *Corbicula fluminea* Growth Reductions Induced by Copper in Artificial Streams and River Systems

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Studies designed to evaluate impacts of copper (Cu) on Asiatic clams, *Corbicula fluminea*, in artificial stream and field environments were conducted. Adult and juvenile responses (survival and growth) to Cu, relevance to Cu bioaccumulation, site-specific comparisons, and the relation to observed densities of resident clams at an impacted site were assessed. Juveniles were more sensitive than adults; however, clam tissue and shell growth were significantly impaired ($p<0.001$) for both juveniles and adults at 8.4–26.7 μg Cu/L in artificial streams. Tissue levels of Cu were 2.5–6 times greater at 8.4–26.7 μg/L exposure concentrations compared with background body burdens in unexposed clams. In studies conducted at the Clinch River, Virginia power plant, clam growth was reduced at 22.5–104.8 μg/L at a water hardness (180 mg/L) that was more than two times the hardness in artificial streams. Clam population densities in the river were well correlated with outcomes of field growth studies. *Corbicula fluminea* growth patterns are a clear and interpretable indicator of Cu contamination in natural and artificial streams and represent a viable alternative to contemporary chronic testing protocols.

Les chercheurs se sont penchés sur l'impact du cuivre (Cu) sur la petite corbeille d'Asie, *Corbicula fluminea*, dans des cours d'eau artificiels et dans le milieu naturel, notamment sur la réponse des adultes et des jeunes (survie et croissance) à Cu, sur l'importance de la bioaccumulation de Cu, sur les comparaisons propre au site et sur la relation aux densités observées de bivalves résidant dans un site contaminé. Les jeunes bivalves étaient plus sensibles que les adultes, mais la croissance des tissus et de la coquille a été gravement perturbée (p inférieur à 0.001) chez les deux à des concentrations de 8.4 à 26.7 μg·Cu/L dans les cours d'eau artificiels. À des concentrations d'exposition de 8.4 à 26.7 μg/L, les teneurs tissulaires en Cu étaient de 2.5 à 6 fois supérieures aux charges corporelles naturelles des bivalves non exposés. Dans des études effectuées dans la rivière Clinch, à la centrale électrique de Virginia, la croissance des bivalves a été ralentie à une concentration de 22.5 à 104.8 μg/L, compte tenu d'une dureté de l'eau (180 mg/L) plus de deux fois supérieure à celle des cours d'eau artificiels. La densité de la population de bivalves dans la rivière affichait une forte corrélation avec les résultats des études de croissance sur le terrain. Le mode de croissance de *Corbicula fluminea* est un indicateur clair et facilement interprétable de la contamination par le cuivre dans les cours d'eau naturels et artificiels et constitue une solution de rechange viable aux protocoles d'épreuves chroniques existants.

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Copper (Cu) is a trace metal contaminant that has received considerable attention by regulatory agencies. The availability of Cu to biota is modified primarily by water hardness (a fact acknowledged for the purpose of developing water quality criteria and standards; e.g. USEPA 1985.) Further, temperature can affect responses of biota by altering metabolic demands and activity.

Compared with effects on estuarine bivalves, effects of Cu on freshwater bivalves has been poorly demonstrated. Only one study of acute Cu toxicity or chronic impairment to freshwater bivalves in the laboratory is included in the 1985 USEPA ambient water quality criteria document for Cu (toxicity to *Corbicula fluminea* by Rodgers et al. 1980) and the data were not included in criteria formulation (USEPA 1985). Data from Rodgers et al. (1980) and Harrison et al. (1984) are at odds with respect to lethal effect concentrations of Cu (96-h LC_{50} of 490 μg/L at 30–50 mg/L hardness in the former versus no mortality after 10 d at 12 000 μg Cu/L and 17 mg/L hardness in the latter). Generally, lower water hardness is associated with increased Cu toxicity and availability. Reasons for these differences are unknown.

The field environment with its inherently variable state, versus the controlled environment of the laboratory, with its unrealistic pristine and simplified design, has created problems in the decision making process for developing water quality criteria. Criteria are formulated primarily based on laboratory evidences of toxicity or impairment. However, conflicts have continued to arise as regulatory agencies assume that laboratory bioassays adequately predict effects on organisms in situ and even for entire ecosystems (Cairns 1983; Kimball and Levin 1985). Laboratory holding/testing requirements for *Corbicula* relative to nutrition, sediment requirements, acclimation, and appropriate test durations that affect toxicity outcomes have recently been addressed by a number of researchers (Graney et al. 1984; Foe and Knight 1985, 1986; Belanger et al. 1986a; Farris et al. 1989). These studies provide the basis to develop field and laboratory test protocols for *Corbicula*.

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Growth rates of *Corbicula* have been shown to be sensitive indicators of stress in the presence of zinc (Zn) and asbestos mineral fibers (Belanger et al. 1986a, b, c). Clams exposed to toxicants in field-located artificial streams have produced data that are more interpretable and less variable than laboratory stream or static system designs. The general utility of *Corbicula* growth patterns as a stress indicator has yet to be determined in uncontrolled but potentially industrially impacted sites in actual receiving systems. If growth rates would indeed index the degree of pollution of the environment, the sensitivity of this easily determined, inexpensive to monitor, but biologically important parameter may allow greater predictions and sound monitoring of water quality reductions from industrial discharges.

Cooling tower blowdown from the Clinch River Plant, operated by the Appalachian Power Company in Southwest Virginia, has been shown to contain potentially toxic levels of Cu (average 857 μg/L). In-stream levels greater than 60 μg/L occur in the first 0.5 km after the discharge enters the Clinch River (Van Hassel and Gaulke 1986). As part of a comprehensive ecological and biochemical investigation of Cu-induced impairment to resident bivalves in this system, the investigation on *Corbicula* growth and mortality responses are reported here. The objectives of this study were (1) to evaluate adult and juvenile *Corbicula* growth and mortality responses to Cu in 30-d field-located artificial stream tests; (2) to relate these changes to Cu accumulation in soft tissues of clams; (3) to repeat (1) and (2) in the Clinch River field setting where a Cu-dominated effluent enters the river, and (4) to compare in situ growth effects by the metal-dominated effluent with population densities of clams in the receiving systems that were not experimentally manipulated.

Materials and Methods

Artificial Stream Studies

Two artificial stream studies were carried out in 1986 near the Glen Lyn Power Plant adjacent to the New River. The first study was in the spring from 29 May to 28 June; and the second in the summer from 5 August to 4 September. In the spring, adult *Corbicula* (14.4–20.8 mm shell length [SL]) were collected from an industrially unimpacted site in the New River at Narrows, Virginia. Clams were transported 13 km to the study site where they were acclimated in oval artificial streams for 4–7 d. The streams used a once-through design with current provided by paddle wheeels driven by a single speed motor (Farris et al. 1989). Coarse sand sediment was added to each stream to a depth of 2 cm. The sediment type and depth was chosen based upon *Corbicula* sediment preference studies by Belanger et al. (1985). New River water, delivered to a headbox via a submerged pump down in the river, entered each of 12 20-L artificial streams at a rate of 1.6 L/min.

Copper was metered into each stream using peristaltic pumps and concentrated stock solutions of $CuSO_4(7H_2O)$. Three replicate streams were randomly assigned at four target concentrations: control (no Cu added), 12, 25, and 50 μg/L. The realized concentrations for the four levels were: 5.1 (background), 16.2, 21.2, and 26.7 μg/L (Tables 1 and 2). Water hardness ranged from 70.6–71.7 mg/L in the streams and temperatures averaged 23.9–24.7°C. Stock solutions were renewed every other day.

The summer experiment was used to evaluate Cu-induced effects on adult (13.15–16.6 mm SL) and juvenile (7.2–9.3 mm SL) clams. Collection and acclimation were as described above. Designation of maturity was based upon methods published by Cherry et al. (1980, 1986) and Doherty et al. (1987). Three replicate streams were used again at the four target concentrations: 0 (control), 6, 12, and 25 μg/L. Realized concentrations were 5.5 (background), 8.4, 13.9, and 17.7 μg/L. Water hardness ranged from 77.3–78.0 mg/L and temperatures averaged 24.8–24.9°C (Table 2).

Growth and Cu Bioaccumulation Analyses in Artificial Streams

Corbicula length and weight gain during artificial stream studies were determined. Baskets constructed of 2 mm^2-mesh were buried in sediment in each stream (one basket per stream) at the beginning of the test. All clams were individually identified and measured for SL by Vernier calipers (± 0.025 mm) and weighed using a Fisher Scientific Model 7301A electronic balance (± 0.5 mg). Individual growth was considered to be the gain in SL and weight from day 0 on successive sampling days. Average growth for each treatment was calculated based upon individuals surviving to that sample day. On days 0, 5, 10, 20, and 30, clams were removed, mortalities counted, outer shells of living clams dried and measured for SL and weight. Baskets were placed in the appropriate streams after clams were measured. Previous studies by Belanger et al. (1986a) using Zn showed that this technique allows tracking of individual clam growth over time.

In the spring study bioaccumulation of Cu was measured in three randomly selected clams (one from each replicate) on days 0, 5, 10, 20, and 30. Copper was measured in algal periphyton and sediment on day 30. Sample preparations by methods modified from Valdes et al. (1982) were used. One sediment sample (particle size <2 mm, $n = 3$ per treatment) and two periphyton samples were scraped from the sides of each stream ($n = 6$ per treatment). In the summer study, periphyton was monitored for Cu accumulation using glass slides (76.2 × 25.2 × 0.85 mm) held in benthic periphytometers. Two slides from each stream were removed on days 5, 10, 20 and 30. Periphyton was scraped from the sides of three streams on day 0 to determine background levels prior to dosing.

In addition to the bioaccumulation analyses for *Corbicula* during the 30-d exposure in the summer study, additional clams were evaluated for evidence of Cu depuration 11 d following the cessation of Cu dosing. Some clams were placed in clean streams after being exposed for 30 d and others were left to recover in contaminated streams. Copper levels (μg Cu/g dry weight) remaining in clams after 11 d and Cu depuration rates (μg Cu/g/d) were calculated.

Clinch River Studies

Two field experiments, to establish the degree of growth inbibition by Cu, were conducted in the Clinch river during 1986. The first was in summer, from 20 August to 18 September at an average water temperature of 22.0°C. The second study was in the fall, from 30 September to 29 October when the river was at 19.6°C (Table 1). Water hardness at a site above the power plant ranged from 155.8–159.2 mg/L during this period (Table 2). The Clinch River is a moderate sized tributary of the Tennessee River and the plant is located approximately 50 km from the headwaters. Therefore, most of the river is shaded from spring through fall. Stations chosen for study were located in shaded sites to reduce temperature fluctuations.

TABLE 1. Overall experimental design of *Corbicula* growth studies during copper exposure in artificial versus natural streams.

Date of study	Location	Life stage	Parameters	Conditions of exposure
29 May to 28 June 1986	Glen Lyn Artificial streams	Adult	—Growth in length, weight —Cu bioaccumulation in periphyton, clams, sediment —clam mortality	5.1, 16.2, 21.1, and 26.7 μg Cu/L
5 August to 4 September 1986	Glen Lyn Artificial streams	Adult Juvenile	—Growth in length, weight —Cu bioaccumulation in periphyton, clams —Cu depuration (recovery) in clams —Mortality	5.5, 8.4, 13.9, and 17.7 μg Cu/L
20 August to 18 September 1986	Clinch River In situ	Adult	—Growth in length, weight —Cu bioaccumulation —Mortality	Two sites above, two effluent affected stations, and two sites below the plant
30 September to 29 october 1986	Clinch River In situ	Adult	—Growth in length, weight —Cu bioaccumulation —Mortality —Field survey of population density	One site above, three effluent affected site stations and one below the plant

In the summer experiment, two stations were located upstream of the plant (1.0 to 3.0 km above the pipe), two stations were directly under the influence of plant discharges (0.025 to 0.5 km below the pipe), and two stations were for potential recovery (0.9–5.5 km below the plant) (Fig. 1). Adult clams (14.1–16.0 km SL) were collected from the New River on 18 August and transported 2.5 h to the Clinch River where they were acclimated for 36 h at the farthest upstream site (station 1). River temperatures were comparable between the New and Clinch Rivers (approximately 23°C). The six sites studied differed sharply with respect to Cu in the water column (Table 2). Concentrations ranged from <15μg Cu/L (stations 1 and 2 [upstream]; stations 14 and 15 [4.1–5.5 km below the plant]) to 104 μg/L (station 8 [0.025 km downstream]).

The fall study was designed to more narrowly define the zones of impact versus recovery based upon the previously collected information. One station was located upstream of the plant (station 2) three stations were directly under the influence of plant discharges (8, 8′ and 11) and one station downstream (15) (Fig. 1). Adult *Corbicula* (13.25–16.20 mm SL) were collected from the New River and transported to the site. New and Clinch River collection temperatures were again similar (19.0 and 17.5°C, respectively). Cu concentrations ranged from <15 μg/L (stations 2 and 15) to 104.8 μg/L (station 8).

Growth, Cu Bioaccumulation and Population Density in the Clinch River

In the summer experiment, 240 clams were weighed, measured, and placed in groups of 10 into nylon mesh baskets as described in artificial stream tests. Four baskets were buried 8 cm into the sediment at each of the six sites. On day 30, baskets were removed and taken to the laboratory. Clams were immediately remeasured for weight and SL and dead clams were enumerated. On day 10, three clams were sampled from predesignated baskets for analysis of Cu uptake and on day 30, five clams were also evaluated for Cu bioaccumulation.

As an index of overall impact of Cu upon clam health, a simple calculation of the production of clams was performed. Production for the population was operationally defined as the net gain or loss of weight from day 0 of all clams that survived to day 30 (i.e. average weight gain of surviving clams × number of surviving clams).

In the fall field experiment, 300 clams were weighed, measured, and placed in groups of 10 in nylon mesh baskets. Six groups of clams were buried at each of the five sites. Three bags were removed on day 10 and the three remaining bags were collected on day 30. Weights and lengths of clams were determined and mortalities counted on each sampling day. Production was estimated on day 30 as described above.

Density of *Corbicula fluminea* at each of the five fall study sites was estimated from three replicate bottom samples, taken by a Surber-type sampler (0.5 m^2, Rodgers et al. 1980). Locations for sampling were selected for similarity of sediment in the field by sieving through 20, 5, and 1-mm mesh sieves. Clams were taken to the laboratory for biomass estimates and analysis of size distribution. Comparison of Cu body burdens were made between clams resident at stations 2 and 15 with the introduced caged clams after their 30 d exposure.

Water Chemistry Analysis

Water samples were collected on days clam growth was measured in artificial stream tests and on a weekly basis in Clinch River studies. Acid soluble Cu concentrations were determined by atomic absorption spectrophotometry using a Perkin-Elmer Model 310 AAS (USEPA 1985). Water samples were initially filtered through a 0.45-μm pore filter and acidified with trace pure nitric acid. Samples were not digested. Total hardness and alkalinity (as mg/L $CaCO_3$) were measured

TABLE 2. Means (± SE in parenthesis below) for selected water chemistry parameters analyzed during *Corbicula* growth experiments at Glen Lyn ($n=6$) and Clinch River ($n=5$).

Study	Station or target Cu concentration (μg/L)	Realized Cu concentration (μg/L)	Temp (°C)	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (μS)
Glen Lyn	0	5.1	24.7	8.44	71.2	46.5	136.0
		(1)	(0.4)	(0.03)	(1.4)	(0.7)	(1.3)
June 1986	12	16.2	24.5	8.31	70.6	45.3	134.7
		(3)	(0.4)	(0.03)	(1.1)	(0.7)	(1.4)
	24	21.1	24.5	8.40	71.7	46.4	134.2
		(2)	(0.4)	(0.04)	(0.7)	(0.7)	(1.5)
	50	26.7	23.9	8.36	70.3	46.9	131.6
		(2)	(0.9)	(0.03)	(0.5)	(0.7)	(1.5)
Glen Lyn	0	5.5	24.8	8.21	77.7	60.1	194.2
		(0.6)	(0.3)	(0.09)	(3.6)	(2.1)	(9.2)
August 1986	6	8.4	23.9	8.21	78.0	59.6	194.9
		(1.1)	(0.3)	(0.11)	(3.1)	(2.1)	(8.9)
	12	13.9	24.9	8.36	77.3	60.4	194.8
		(2.2)	(0.3)	(0.05)	(3.3)	(2.1)	(9.1)
	25	17.7	24.9	8.18	78.0	60.8	195.3
		(2.5)	(0.3)	(0.08)	(3.1)	(2.2)	(9.0)
Clinch River[a]	1	<15.0	22.4	8.40	159.2	144.8	298.5
			(0.6)	(0.09)	(7.1)	(7.1)	(5.3)
August –	2	<15.0	22.6	8.45	155.8	156.0	299.9
			(0.8)	(0.05)	(5.4)	(9.1)	(7.0)
October 1986	8	104.8	21.8	8.36	186.6	117.0	366.2
		(18.9)	(1.2)	(0.03)	(13.4)	(5.9)	(19.5)
	11	47.4	22.3	8.17	185.0	142.6	360.6
		(11.0)	(1.2)	(0.21)	(8.8)	(13.3)	(16.3)
	14	22.5[b]	22.7	8.41	160.2	164.8	320.5
		(0.3)	(0.8)	(0.14)	(5.6)	(12.2)	(7.9)
	15	<15.0	22.6	8.32	165.8	159.6	323.7
			(0.7)	(0.16)	(4.3)	(10.6)	(7.1)

[a]Combined data for the 2-mo period. Note that stations 8′, 13, and 16 were not evaluated for water chemistry.

[b]Three of five measurements were < 15.0 μg Cu/L.

titrimetrically, conductivity was determined by a YSI Model 33 meter, and pH by an Ultrex Model 3560 pH meter. Stream temperatures were monitored at least twice weekly.

Statistical Methods

Statistical analysis of length and weight gain, Cu bioaccumulation, and population density estimates consisted of one-way analysis of variance (Bhattacharya and Johnson 1977) by SAS GLM procedures (SAS 1985) on each sample day. Thus for artificial stream experiments the experimental design consisted of four Cu treatments replicated three times (dependent variable) with growth or bioaccumulation response (independent) variables analyzed on each sample day independently. Similarly, in field tests, site (dependent variable) locations were controlled and growth, bioaccumulation, and population density were response variables. Duncans New Multiple Range Test was used to detect significantly different means ($\alpha = 0.05$) if the ANOVA was significant. Student *t*-tests were used to evaluate differences in Cu body burdens in resident clams collected in the field versus those held in cages for 30 d. Significance was inferred at $p<0.05$. When the data structure was appropriate, 30-d LC_{50} mortality estimates in responses to Cu were made using the Trimmed Spearman-Karber Method to aid comparisons with previously published studies (Hamilton et al. 1977).

Results

Corbicula Responses to Cu in Artificial Streams

In the spring artificial stream test, all treatment exposures significantly reduced shell growth (Fig. 2A). By day 10, shell length and weight gain (Fig. 2B) was significantly reduced at 16.2–26.7 μg/L. Control clams exhibited more than 20-fold greater shell growth than clams exposed to Cu. Clams exposed to the highest Cu concentration lost shell mass by day 30 (termed degrowth by Russell-Hunter 1985). Substantial mortality (10–70%) was observed by day 20 in Cu exposures with nearly 100% mortality at 26.7 μg/L on day 30 (Table 3). A 30-d LC_{50} of 19.2 μg/L (95% confidence interval of 18.5–19.9) was calculated.

During the spring experiment Cu accumulations appeared to increase linearly with time in clams and failed to stabilize at any concentration (Fig. 2C). All exposures resulted in comparable tissue burdens (205–278 μg Cu/g dry weight). Copper content of controls remained constant at 40–60 μg Cu/g dry weight.

During the summer experiment Cu exposure concentrations were reduced in an effort to determine a threshhold below which Cu would not affect *Corbicula*. Exposures of 13.9 and 17.7 μg/L reduced adult clam shell growth by day 20 and 10, respectively (Fig. 3A). Clams exposed at 8.4 μg/L grew only 50% of that observed in controls, but were not significantly

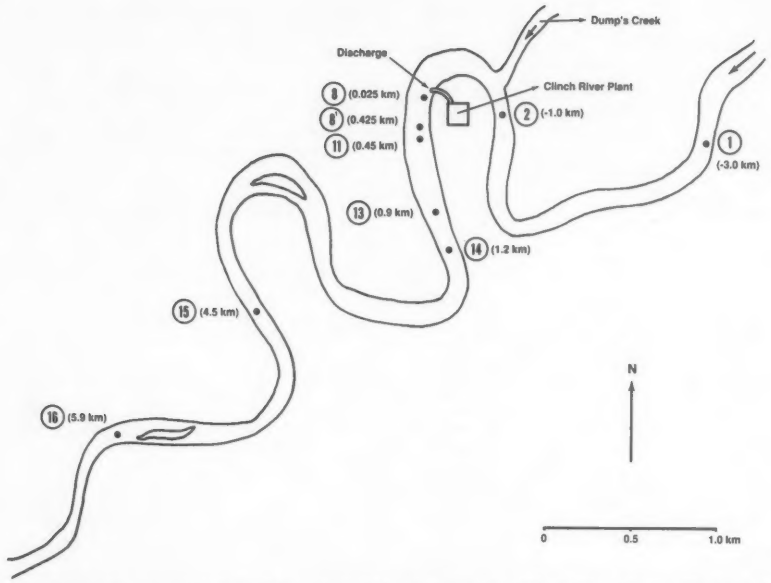

FIG. 1. Schematic representation of the relationships between the Clinch River Power Plant cooling tower discharge, Clinch River Plant, and stations where *Corbicula* growth and population density estimates were collected.

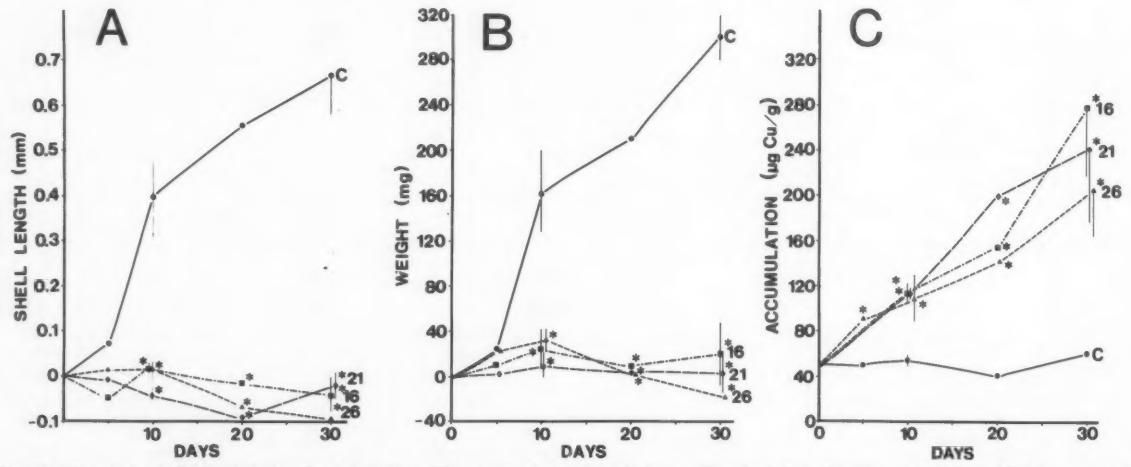

FIG. 2. Growth in shell length (A) and weight (B) and Cu accumulation in soft tissues (C) of adult *Corbicula* exposed in artificial streams at Glen Lyn (May–June 1986). Asterisks represent significantly different means from controls. On days 10 and 30, indications of variability (± 1 SE) are given. Numbers next to lines indicate exposure concentration.

TABLE 3. Mortality of adult and juvenile *Corbicula* after 30 d of exposure to copper in artificial streams at Glen Lyn.

		Percent mortality at each Cu concentration (µg/L)						
Study	Lifestage	5.1	8.4	13.9	16.2	17.7	21.1	26.7
Spring	Adult	0	—	—	25.0	—	59.4	96.7
Summer	Adult	0	0	13.3	—	63.3	—	—
Summer	Juvenile	0	0	43.3	—	100.0	—	—

different. Weight gain was not significantly affected until day 30, but was reduced at all concentrations (Fig. 3B). Mortality was comparable to the previous study with approximately 60% dead at 17.7 µg/L by day 30. A 30-d LC_{50} statistic could not be calculated.

The summer rate of bioaccumulation of Cu occurred at about the same rate as in spring, but appeared to reach a plateau between days 20 and 30 (Fig. 3C). Stream temperatures between studies differed by only a few tenths of a degree reducing the potential confounding effect of temperature on bioaccumulation rates (Table 2). Clams were allowed to depurate under two

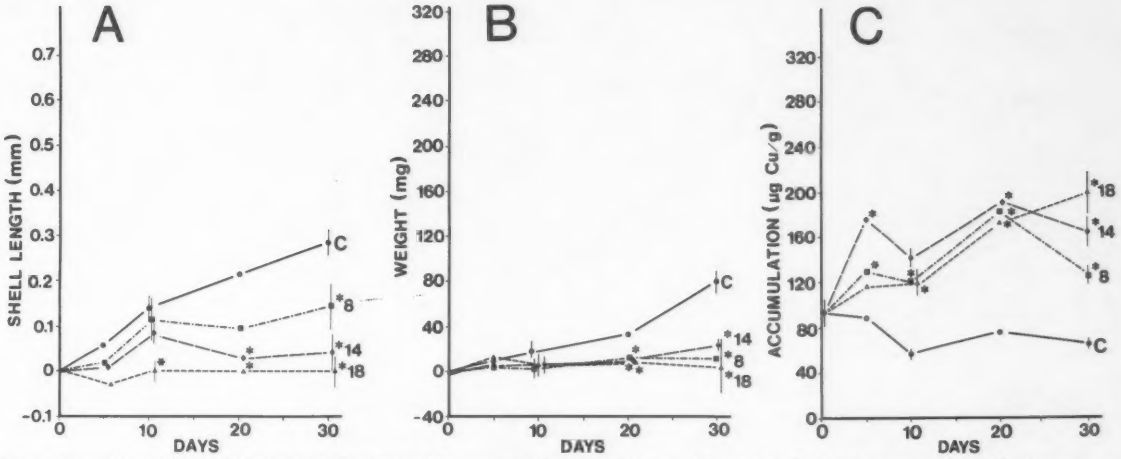

FIG. 3. Growth in shell length (A) and weight (B) and Cu accumulation in soft tissues (C) of adult *Corbicula* exposed in artificial streams at Glen Lyn (July–August 1986). Numbers next to lines indicate exposure concentration.

TABLE 4. Copper depuration in clams previously exposed to copper in artificial streams (5 August to 4 September 1986). Clams were held in previously Cu-contaminated and in uncontaminated streams. Measurements were made on three clams from each treatment on day 30 (no depuration) and 41 d (11 d depuration). Significantly different means from the control on day 30 are indicated by an asterisk (* = 0.05).

		Contaminated streams		Uncontaminated streams	
Previous Cu exposure level (µg/L)	Bioaccumulation by day 30 (µg/g)	Cu body burdens on day 41 (µg/g)	Rate of loss ($\mu g \cdot g^{-1} \cdot d^{-1}$)	Cu body burdens on day 41 (µg/g)	Rate of loss ($\mu g \cdot g^{-1} \cdot d^{-1}$)
5.5	80.4 ± 11.1	—	—	—	—
8.4	124.4 ± 13.6*	121.2 ± 3.9*	0.3 ± 0.4	98.4 ± 9.3	2.4 ± 0.8
13.9	170.7 ± 16.4*	120.6 ± 8.2*	4.6 ± 0.8	89.6 ± 11.3	7.3 ± 1.0
17.7	205.1 ± 21.3*	136.8 ± 11.1*	6.2 ± 0.9	119.6 ± 14.2*	7.8 ± 1.3

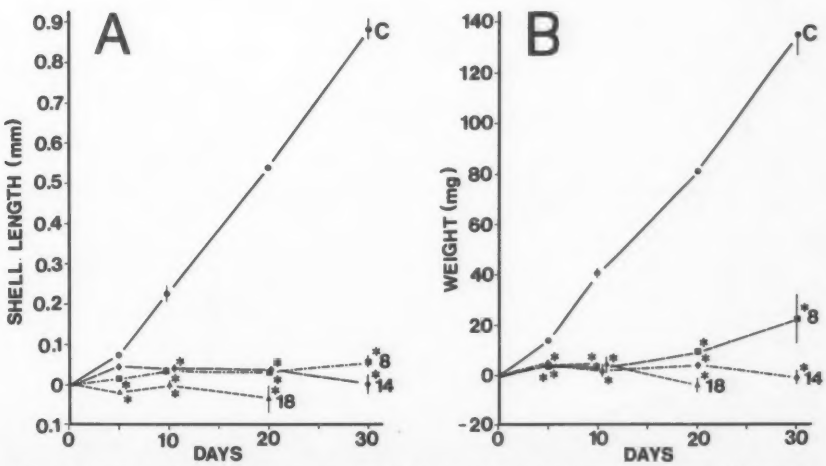

FIG. 4. Growth in shell length (A) and weight (B) in juvenile *Corbicula* exposed in artificial streams at Glen Lyn (July–August 1986). Numbers next to lines indicate exposure concentration.

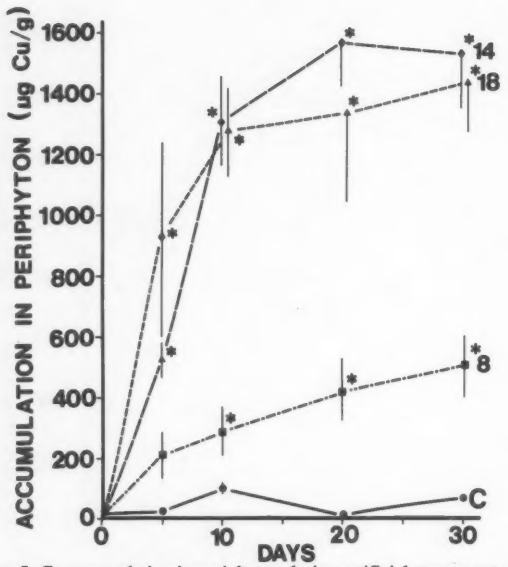

FIG. 5. Cu accumulation in periphyton during artificial stream tests at Glen Lyn (July–August 1986). Numbers next to lines indicate exposure concentrations.

conditions for 11 d after Cu application ended. Clams maintained in streams that had previously become contaminated responded with slower Cu release rates than those removed to uncontaminated streams (Table 4). Rates of loss were highest (7.8 µg Cu depurated·g^{-1}·d^{-1}) for clams that previously had the greatest burdens (205.1 µg Cu/g). At the intermediate Cu exposure levels, body burdens returned to near control levels (80.4 µg Cu/g) in uncontaminated streams, but remained elevated or substantially unchanged in contaminated streams.

Juvenile *Corbicula* exposed in artificial stream 30-d tests had significantly reduced shell growth at 8.4 and 17.7 µg/L by day 5 and at all exposure concentrations by day 10 (Fig. 4A). By day 20, clams exposed to 17.7 µg/L exhibited degrowth and did not survive to day 30. Similar effects were found for weight gain where all juveniles exposed to Cu had negligible growth over 30 days (Fig. 4B). A 30-d LC_{50} for juvenile clams was 13.4 µg/L (95% confidence limits of 12.9–13.9 µg/L).

Accumulations of Cu in algal periphyton were measured more frequently during the summer study (Fig. 5). Accumulation was rapid, but stabilized between days 10 and 30. Peak concentrations were measured at 1530 µg Cu/g dry weight at 13.9 µg/L on day 20, although little difference was observed between the higher treatment levels. At 8.4 µg/L, the lowest concentration tested, approximately 400 µg/g was observed by day 30.

Corbicula Responses to a Cu-dominated Effluent

The farthest downstream station (16, 5.5 km below the plant) was most hospitable for *Corbicula* as clams grew the most in length and weight (Fig. 6A, B). By station 15 (4.1 km below the outfall) Cu was below 15 µg/L (Table 2). Clams placed above the plant (i.e., controls at stations 1 and 2) had significantly greater growth than stations 8 (104.8 µg/L, immediately below the discharge) and 11 (47.4 µg/L, 0.3 km below the discharge) and potential recovery station 14 (23 µg/L, 0.9 km below the discharge). Of these latter stations, station 11 showed the highest impact as clams had shell degrowth and negligible weight gain. The greatest mortality occurred at station 8 (32.5%). Stations 1, 2, 11, 14 and 16 had 5.0, 15.0, 17.5, and 0% mortality, respectively.

Estimates of production integrated mortality and growth evaluations. Because the production was calculated for an entire population, variance estimates are not possible to derive from unreplicated measurements. The greatest production occurred at station 16, 5.5 km below the plant (Table 6). At the two upstream sites, production was very similar (3944 mg at station 2 versus 4750 mg at station 1). Stations 8 (immediately where the discharge entered the river) and 11 (0.5 km below the pipe) had productivity that was 73 and 91%, respectively, less than control sites. Clearly, *Corbicula* was affected at these locations.

Clams held at station 8, 11, and 14 contained twice the level of Cu on day 10 as those at stations 1 and 2 (the station 16 sample was lost during the transfer from field to the laboratory). By day 30, clams at station 11 contained 161.5 versus 40–50

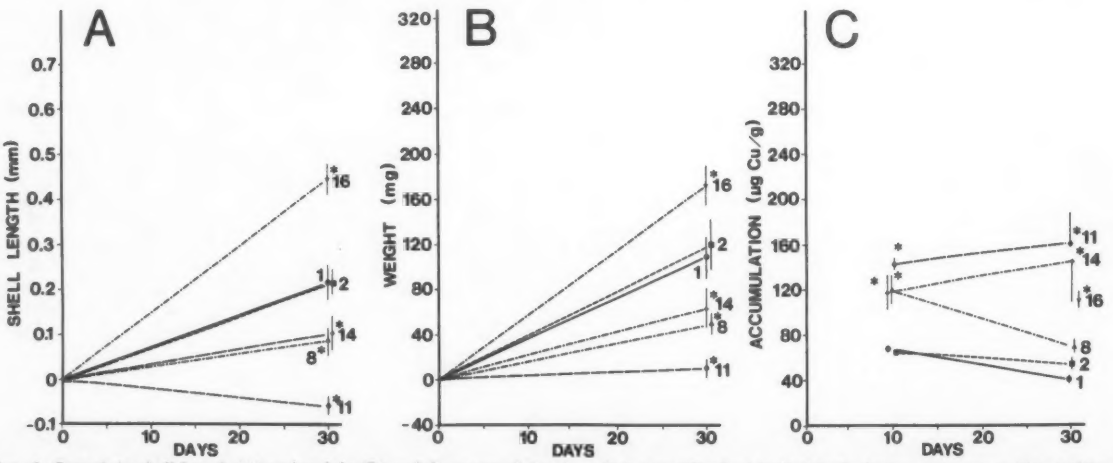

FIG. 6. Growth in shell length (A) and weight (B) and Cu accumulation in soft tissues (C) of adult *Corbicula* during exposure to Clinch River Plant Cu discharges in the Clinch River (August–September 1986). Number next to lines indicate exposure location.

TABLE 5. Bioaccumulation (mean ± SE) of copper (Cu) by algal periphyton and accumulation in sediment by day 30 in Glen Lyn artificial streams. The exposure period was from 29 May to 28 June 1986. Significantly different means from the control are indicated by an asterisk (* = 0.05). Sample size (n) was 6.

Cu exposure concentration (μg/L)	Cu concentration in algae (μg Cu/g dry weight)	Cu concentration in sediment (μg Cu/g dry weight)
5.1 (control)	50.7 ± 1.6	6.1 ± 2.3
16.2	513.5 ± 85.7*	22.2 ± 4.3*
21.2	1248.9 ± 98.8*	40.1 ± 21.7*
26.7	1556.1 ± 243.2*	37.4 ± 1.5*

TABLE 6. Estimates of total production of *Corbicula* populations exposed to Clinch River River power plant effluent in the field measured as weight gain by all surviving clams.

Station	Distance from discharge pipe (km)	Weight gain for all surviving clams (mg wet weight/population)
	Summer	
1	−3.0	4750
2	−1.0	3944
8	0.025	1161
11	0.5	429
14	0.9	2520
16	5.5	6270
	Fall	
2	−1.0	2528
8	0.025	−390
8′	0.4	−504
11	0.5	−21
15	4.1	1378

μg/g at the upstream sites. Significantly elevated levels of Cu were found in clams from sites as far downstream as 5.5 km below the discharge.

In fall, five locations were chosen for further field testing. Clams held at stations 8, 8′ and 11 had significantly less shell growth than at stations 2 and 15 (Fig. 7A). Temperatures averaged 17.5 versus 20°C for the summer study. Clams at station 2 had significantly greater weight gain than all other sites including station 15 (Fig. 7B). Degrowth in weight was observed at 8, 8′ and 11 (0.025–0.5 km below the pipe). Clam mortalities (from upstream to downstream) on day 30 were 10.0, 13.3, 6.7, 0, and 0% for stations 2 through 15, respectively.

Production of clams at stations 2 (1 km above the plant) and 15 (4.1 km below the plant) were comparable (within a factor of 2). However, clams at stations 8 (0.025 km below the pipe) to 11 (0.5 km below the pipe) had negative production, reflecting the overall negative growth and mortality at these sites. The absolute magnitude of production cannot be compared between summer and fall studies because of differences in samples size (40 versus 30, respectively), but trends were similar.

Copper bioaccumulation was similar to the previous study, with a maximum of 205 μg/g dry weight at station 8 on day 30 (Fig. 6C). Copper accumulation was at the same level at all stations located downstream of the plant compared with the reference station 2 (46.4 μg/g). Similarly, native clams collected from the Clinch River at station 2 had low Cu loads (14.5 + 2.1 μg/g, $n = 5$) compared with those from station 15 (115.9 + 17.3 μg/g, $n = 4$). T-tests of the effect of clam source on Cu content showed that no significant differences for clams collected (resident) or placed (experimental) at station 15 ($p > 0.25$), but at station 2, resident clams had significantly lower Cu levels in soft tissues ($p < 0.005$).

Corbicula density, biomass, and size distributions were estimated at each site (plus a sixth, station 13, Fig. 1) at the beginning of the fall field study. Densities of clams collected at stations 2 (1 km above the plant) and 15 (4.1 km below the plant) were significantly greater than at 8, 8′, and 11 (0.025–0.5 km below the plant) (Table 7). However, station 13 (0.6 km below the plant) was not significantly different from 8, 8′, and 11 (no clams present at all) and had densities that were approximately one-third those of station 2. Biomass at stations 2, 13, and 15 were 12.085, 1.969, and 13.773 g total weight/m^2. Clams from station 2 were significantly larger than those from station 13, but not 15.

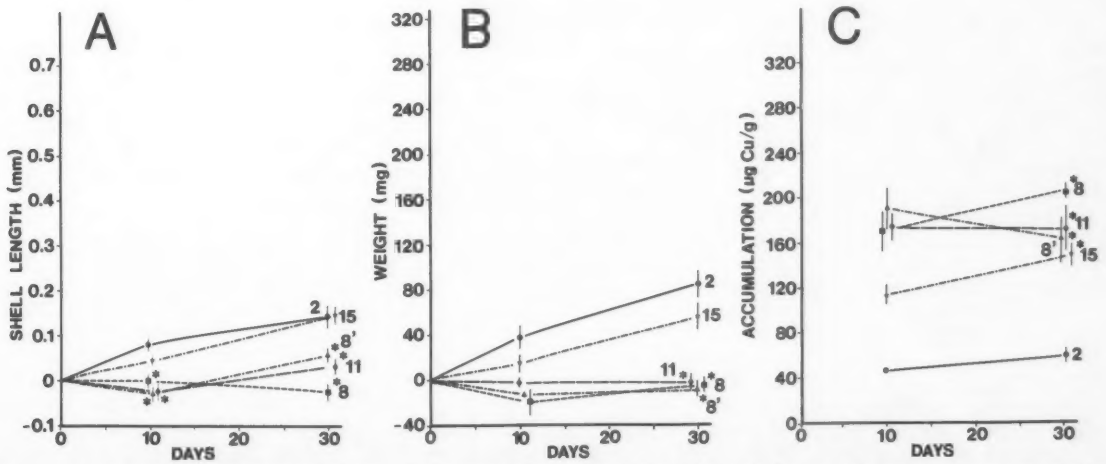

FIG. 7. Growth in shell length (A) and weight (B) and Cu accumulation in soft tissues (C) of adult *Corbicula* during exposure to Clinch River Plant discharges in the Clinch River (September–October 1986). Number next to lines indicate exposure location.

TABLE 7. *Corbicula* density, biomass, and size at selected sites near the Clinch River Plant on 29 September 1986. Significantly different means are indicated by different superscript letters as determined by Duncan's Multiple Range Test ($p = 0.05$).

Station	n	Density (clams/m^2)	Biomass (g/m^2)	Shell length (mm)
2	42	14.2 ± 9.2^{a}	12.085	10.51 ± 0.76^{a}
8	0	0^{b}	—	—
8′	0	0^{b}	—	—
11	0	0^{b}	—	—
13	16	$5.3 \pm 2.4^{a,b}$	1.969	7.45 ± 0.97^{b}
15	58	19.3 ± 8.8^{a}	13.733	$9.38 \pm 0.71^{a,b}$
ANOVA				
F-ratio		3.58	—	5.72
p-value		$p<0.05$	—	$p<0.01$

Discussion

Growth and Mortality of Clams in Artificial Streams

Freshwater bivalves have not been well incorporated into decisions regarding water quality criteria and the protection of water and its uses. Much of the lack of emphasis may be attributed to the difficulties associated with collecting and maintaining freshwater bivalves in the laboratory (resulting in few available test species), insufficient knowledge of the sensitivity of bivalves to toxics, and the paucity of data on in-stream effects of toxicants to populations of bivalves in the field. In the controlled artificial stream studies presented here, all levels of Cu reduced one or both of shell length and tissue growth in 30 d. The spring artificial stream study did not include as low an exposure level as that in the summer study (8.4 μg/L), but the two studies varied only slightly in temperature (0.5–1.0°C difference) between seasons.

It is quite clear that *Corbicula* growth is a highly sensitive parameter for evaluation of metal-induced impacts. Shell degrowth observed in adults occurred at 16–26 μg/L in artificial streams. This is probably a direct result of reduced siphoning activity by clams even though suspended periphyton (the primary food source) was quite abundant in all streams. MacMahon and Williams (1986) recorded degrowth in *C. fluminea* during low winter temperatures. Belanger et al. (1986b, c) showed reduced growth or degrowth during 30-d exposures to asbestos mineral fibers was accompanied by significantly reduced siphoning activity. In the studies reported here growth was affected as early as 10 d; however, extending exposures to 20- and 30-d increased reliability through repeated measurements.

Juvenile clams wre more sensitive to Cu than adult clams. Juveniles were eliminated at 17.7 μg/L and exhibited no growth even at the lowest concentration (8.4 μg/L). Doherty and Cherry (1988) in a comprehensive literature review of the *Corbicula* acute toxicity literature found little evidence for stage-dependent sensitivity as did Belanger et al. (1986a) when evaluating Zn. Harrison et al. (1984) compared adult and veliger *Corbicula* sensitivity to Cu. They found adults suffered 55% mortality at 11 μg/L in water of 17 mg/L hardness after 30 d; whereas, veligers had 53% mortality in 24-h at 27.4 μg/L. Although the water hardness in artificial streams was substantially greater in the present study (71–78 mg/L), 30-d LC_{50}s for adults (19.2 μg/L) and older juveniles (13.4 μg/L agree well with Harrison. The need for further investigation of the sensitivity of juvenile clams to Cu is clearly indicated.

Bioaccumulation of Cu in Artificial Stream Tests

Copper in clams exposed to 8.4–26.7 μg/L resulted in significantly greater concentrations than controls. Although strict quantitative comparisons at each level is difficult due to limited numbers of samples, certain trends are evident. First, body burdens of Cu, especially at higher exposure concentrations, were associated with reduced growth or degrowth in *Corbicula*. Bioconcentration factors (BCF) for these data ranged from 20 567 at 16.2 μg/L to 8119 at 26.7 μg/L in the spring study and 7000 at 13.9 μg/L to 7345 at 17.7 μg/L in the summer study. Graney et al. (1984) determined BCFs of 8600 at 57 μg/L and 15 896 at 16 μg/L in 28-d tests with *Corbicula* from the New River.

The predominant source of trace metals for clams in these artificial stream studies is probably suspended periphyton sloughed from walls and sediment (Belanger et al. 1986a; Genter et al. 1987). Coper in algal-periphyton within our streams had up to 1500 μg Cu/g at 13.9–17.7 μg/L. It is most probable that suspended algae ingested by clams is derived primarily from periphyton sloughed from walls in the streams and is a major route of Cu uptake by clams.

Variation in mean levels of Cu in clams was observed. Such variability may be explained by altered siphoning in response to the presence of Cu resulting in fluctuating body burdens of Cu. Doherty et al. (1987) found clams were able to detect levels of metals and chlorine at or below levels that were acutely toxic. After clams close, sometimes for several hours, energetic demands of the individual are not eliminated, therefore, shell or tissue degrowth can proceed. Belanger et al. (1986b) and Sappington (1987) described this phenomenon for asbestos and biocides other than chlorine and it appears to be a generalizable response to stressors. That this indeed occurs makes it extremely difficult to interpret bioaccumulation patterns and the relation to toxicity or long-term impairment of the bivalve.

Previous studies by Belanger et al. (1986a) with Zn showed that once growth inhibition was induced, the reductions were long-lasting. After Zn exposure was stopped, clams retained size differences established during Zn exposure while clams depurated Zn and returned to normal growth rates within 10–15 d. Body burdens of Zn were completely eliminated within 2 wk after exposures were halted. In this study, Cu body burdens in adult clams were evaluated 11 d after exposures were stopped. Clams were maintained in streams that never received Cu inputs. Clams in contaminated streams had lower Cu loss rates than those in clean streams, probably due to the presence of Cu in algae (500–1500 μg/g) and sediment (20–40 μg/g). The slow rates of Cu loss in *Corbicula* in the contaminated streams underscore the role of food (algae) and sediment contamination to filter feeding bivalves and their probable impact on growth.

Growth and Mortality of Clams in the Clinch River

The Cu-dominated effluent of the Clinch River Power Plant had a significant impact on clams when levels of Cu were 47.4 to 104.8 μg/L. Evidently, however, the higher levels of water hardness in the Clinch River (approximately 160 mg/L) compared with the New River (approximately 80 mg/L) substantially reduced the level of acid-soluble Cu available to inhibit clam growth. In the softer water of the New River, levels of Cu at or above 8.4 μg/L reduced clam growth by 50%. In the Clinch River, clams exposed to three times that concentration (22.5 μg/L) had a similar percent reduction in growth. When in-

stream concentrations were higher (>47 μg/L) growth was completely inhibited.

Clams showed responses to lower concentrations (only 13.8 μg/L) in artificial streams. Two reasons are probably responsible. The first is a two-fold increase in hardness for the field studies. The second is the regulated nature of the artificial streams, where scouring and water levels are tightly controlled. In the Clinch River, freshets may drastically alter the clam habitat by flushing suspended algae and contaminated periphyton food sources from contaminated reaches of the river. Suspended sediment loads in the river are also altered by freshets and may change biologically available Cu concentrations by binding.

Under field conditions, clams exposed to effluent did not experience as much mortality as may have been suggested by growth reductions when compared with artificial stream tests. In the controlled conditions, clams experienced similar growth depressions with higher mortality that sometimes exceeded 60% at 20 μg/L or greater after 30 d. These differences may be associated with temperatures at which tests were conducted. For example, Glen Lyn artificial stream experiments were conducted at 23–25°C, but the fall Clinch River study was performed at 17.5°C. Those clams under the most severe conditions in the river (104.8 μg/L) experienced degrowth and only 13.3% mortality. At 17.5°C, clams may siphon less even in the absence of toxicant than at higher temperatures resulting in lower energetic demands. At the same time, sensitivity to toxicants will be less (Doherty and Cherry 1988). At station 8 in the summer, clams suffered 32.5% mortality at 23°C.

Production calculations for the various exposed clam populations can be used to integrate the effects of Cu on mortality and growth of individuals. It is clear that 0.025–0.5 km below the discharge (104.8–47.4 μg/L), clams were impaired. Negative or only slightly positive production (− 504 to 882 mg wet weight gained per population of 30 clams) was encountered at these sites. This comparative method shows promise for future studies.

Bioaccumulation of Cu in the Clinch River

Copper uptake in clams held in the field showed that clams held in effluent-impacted sites (0.025–0.50 km below the discharge) accumulated Cu to levels similar to downstream, unimpacted sites (0.9–5.5 km below the discharge). Considerable differences in water-borne Cu were measured in these two reaches with > 47.4 μg/L in the former group, versus 22.5 μg/L and less in the latter. That these reaches had divergent growth results, but not distinctive bioaccumulation patterns, suggests that extreme care in using bivalve accumulation patterns to assess metal contamination must be used. At station 8 in the summer field study, clams apparently lost some metal burden between day 10 and 30. This nonconformity may reflect some change in siphoning activity, but at best this is speculative.

Copper body burdens in clams collected from sites 2 and 15 in the field were compared with caged clams and showed that at station 15 resident clams had similar burdens as caged clams, but at station 2 resident clams had significantly less Cu than caged clams. A potential contributing factor to this discrepancy might be that New River *Corbicula* are in a drainage that receives more Cu inputs than the Upper Clinch River. This is supported by some measurements of Cu in artificial streams at Glen Lyn, even in the control (no Cu added) condition. The carry over of some Cu body burdens in transplanted clams in the Clinch River studies would account for elevated levels in caged clams.

Evaluation of Clam Density in the Clinch River

Differences in densities of *Corbicula fluminea* observed in the field correspond well with changes in clam growth. At sites where little or negative growth occurred (0.025–0.5 km below the pipe) no naturally occurring clams were found. Production estimates were an order of magnitude or less lower than in sites further downstream of the plant (0.9 km or more) or control sites above the plant. Further, downstream sites that were unaffected in clam growth studies (particularly station 15) had densities that were not significantly different from the upstream control stations. One downstream site (station 13, 0.6 km below the pipe) did have clams present, but at densities substantially reduced from control sites. This would have been predicted from *Corbicula* growth data because clams at station 14 (0.9 km below the plant) were impacted by effluent according to the growth studies.

Classically, laboratory investigations have formed the core of knowledge used to predict impacts of chemicals on real world environments (Branson 1978). In the past few years, it has become increasingly common to "validate" laboratory data on field impacted situations (Cairns 1988), especially through the use of environmentally realistic laboratory artificial stream designs. In the controlled artificial stream environment, responses of clams to Cu (or other perturbants) can be evaluated in a rigorous fashion and give reasonable/reliable estimates of exposure effects. Artificial stream and river transplant growth studies are insightful in explaining the absence of clams in sites near the Clinch River plant discharge (0.5 km or less distant from the pipe) as well densities and sizes of *Corbicula* marginally affected below the outfall.

Our results accord well with other in-stream studies at the Clinch River plant that have been performed to determine impacts of effluent discharges. Significantly, time-intensive macroinvertebrate monitoring studies (Van Hassel and Gaulke 1986) agree well with biochemical indices of stress in *Corbicula* (e.g. the cellulolytic activity index, Farris et al. 1988). Cellulolytic activity was significantly depressed when Cu met or exceeded 22 μg/L in the field. Growth is undoubtedly influenced by the ability of clams to degrade ingested algal foods and in combination with cellulase enzyme activity analysis, growth will prove to be a useful tool to evaluate the influence of effluents or toxicants to bivalves. Growth studies of this nature will ultimately be useful in a regulatory framework where impact of discharges on freshwater bivalves has been ignored or underdeveloped because of the lack of suitable models.

Acknowledgements

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Aggressiveness and the Foraging Behaviour of Young-of-the-Year Brook Charr (*Salvelinus fontinalis*)

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Grant, J. W. A. 1990. Aggressiveness and the foraging behaviour of young-of-the-year brook charr (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 47: 915–920.

Individual young-of-the-year brook charr (*Salvelinus fontinalis*) were observed in the field to relate their aggressiveness to their body size, the size and quality of their foraging site, and their feeding rate. Aggressive charr were 13% larger than nonaggressive conspecifics observed on the same day in the same area. Aggressive fish had a lateral foraging radius 29% larger than nonaggressive fish and a 45% greater mean distance to neighbours, even when the effect of body size was removed by analysis of covariance. Aggressive fish of two size categories (20–29 and 40–99 mm) made 70 and 55% more feeding attempts per unit time, respectively, than nonaggressive fish of the same size. Number of prey items in stomachs was significantly and positively correlated with feeding attempt rate. In addition, aggressive fish appeared to occupy more preferred sites, since their sites were significantly more likely to be reoccupied after their removal than were sites occupied by nonaggressive fish. These results are consistent with the hypothesis that defence of a foraging site is a better tactic than ignoring conspecifics.

Quelques ombles de fontaine de l'année (*Salvelinus fontinalis*) ont été observés individuellement sur le terrain afin de relier leur agressivité à leur taille corporelle, à l'étendue et à la qualité des aires d'alimentation et au rythme d'alimentation. Les ombles agressifs avaient une taille de 13 % supérieure à celle des congénères non agressifs observés le même jour dans la même région. Les poissons agressifs avaient un rayon de d'alimentation latéral supérieur de 29 % à celui des poissons non agressifs et gardaient une distance moyenne vis-à-vis de leurs voisins de 45 % supérieure, même lorsque l'effet de la taille corporelle était éliminé par analyse de covariance. Les poissons agressifs de deux catégories de taille (20–29 et 40–99 mm) faisaient respectivement 70 et 55 % plus de tentatives d'alimentation par unité de temps que les poissons non agressifs de même taille. Le nombre de proies trouvées dans les estomacs des poissons a été corrélié de façon significative et positive au rythme des tentatives d'alimentation. En outre, les poissons agressifs semblaient occuper davantage les sites préférés, puisque leurs sites étaient beaucoup plus susceptibles d'être occupés de nouveau après qu'ils les aient abandonnés que les sites occupés par les poissons non agressifs. Ces résultats concordent avec l'hypothèse selon laquelle il est préférable de défendre l'aire d'alimentation que de ne pas tenir compte des congénères.

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Salmonid populations in streams are often assumed to be limited by the space available for the feeding territories of juveniles (Chapman 1966; Allen 1969). Individuals not acquiring a territory are presumably displaced downstream (Chapman 1962; Mason and Chapman 1965) or die (Elliott 1986). According to this interpretation, territoriality is the best feeding tactic; nonterritorial individuals are trying to "make the best of a bad job" (Dawkins 1980).

Support for this interpretation has come primarily from laboratory experiments where fish are confined at higher than normal densities. In these experiments, body size and dominance status are positively related (Newman 1956; Chapman 1962; Symons 1971; Noakes 1980; Ferguson and Danzmann 1985; but see Bachman 1984 for field data), and upstream positions are typically occupied by larger (Mason and Chapman 1965; Mason 1969; Noakes 1980) or more dominant (Reimers 1969; Gibson 1983; Fausch 1984) individuals, who have priority of access to drifting food. Dominant fish grow faster than subordinates in such conditions (Chapman 1962; Fenderson et al. 1968; Symons 1971; Li and Brocksen 1977; Fausch 1984).

Surprisingly, there is little evidence from the field to confirm that territoriality, or its presumed correlates such as size or aggressiveness, increases either the foraging rate or growth rate of individual salmonids. Jenkins (1969) and Bachman (1984) found no correlation between dominance rank and feeding rate of brown trout (*Salmo trutta*), whereas McNicol et al. (1985) reported that aggressive brook charr (*Salvelinus fontinalis*) fed at a lower rate than nonaggressive fish. The one notable exception is the study of Puckett and Dill (1985) who reported that the cost per feeding attempt was lower for territorial coho salmon (*Oncorhynchus kisutch*) than for either nonterritorial fish or for the 'floaters' that exist in the spaces between the territories of other fish. However, their data were less clear about the foraging benefits of aggressiveness; nonterritorial fish had the lowest feeding rate, but there was no significant difference between the feeding rate of territorial fish and floaters.

Brook charr in streams may defend exclusive territories, defend partial territories against smaller intruders only, or tolerate conspecifics (Keenleyside 1962; McNicol and Noakes 1981, 1984; McNicol et al. 1985). Previous papers reported the influence of current velocity on variation in aggressive behaviour and mobility of brook charr (Grant and Noakes 1988) and compared the sizes and shapes of territories and foraging zones

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(Grant et al. 1989). In this field study, I quantify the relation between aggressiveness and foraging benefits of young-of-the-year brook charr. Specifically, I test the prediction that there is a positive relation between the aggressiveness of individual brook charr and their body size, foraging site quality, and feeding rate.

Methods

Young-of-the-year brook charr were observed between April 1 and August 31 during 1984, 1985, and 1986 in three streams near Guelph, Ontario (see Grant and Noakes 1988 for a description). I made focal animal observations of fish while sitting motionless on an elevated seat 1–2 m downstream of the focal fish or while sitting or lying on the stream bank. Brook charr in running water typically hold position in the stream and return to the same position after each feed or chase. A focal point was defined as the snout of the fish at this location.

The feeding rate of focal fish was quantified during a 15-min observation. I could not always see the prey item and the fish did not always eat the captured item. Hence, all feeding attempts were counted whether successful or not. The location of a feeding attempt was defined as the place where the mouth snapped closed. Distances of feeding attempts were estimated to the nearest half body length from the focal point. Directions were recorded as 1–12 o'clock, with the upstream direction as 12 o'clock. Lateral foraging radius was defined as the mean distance of feeding attempts to the side (2–4 and 8–10 o'clock directions).

I monitored the reaction of the focal fish to intruders during the 15-min observation and for an additional 15 min, except for some mobile fish that were more difficult to follow for this length of time. I defined intruders as conspecifics that approached to within two body lengths of the focal point from the front or side (9 to 12 and 12 to 3 o'clock directions) or that elicited an agonistic response. Only fish approached by three or more intruders were included in the analyses to ensure that all fish had an opportunity to respond to intruders. The response of the focal fish was recorded as overt responses (charge, chase, nip, approach), lateral displays, no response, and withdrawal. Focal fish were classified as: (1) aggressive, if they overtly responded (at least three times) to at least 50% of intruders; (2) occasionally-aggressive, if they overtly responded to less than 50% of intruders; and (3) nonaggressive, if they did not use overt responses to intruders. Many focal fish could not be categorized with respect to aggressiveness because of low intrusion rates. I did not classify fish as territorial or not, because there appeared to be a continuum among individuals in their aggressive behaviour rather than a sharp threshold. However, individuals that I classified as aggressive and nonaggressive roughly correspond to territorial and nonterritorial/floater, respectively, of Puckett and Dill (1985). Occasionally-aggressive is probably equivalent to fish that defend 'partial territories' against smaller intruders only (Greenberg 1947).

Neighbours were defined as any conspecific that assumed a stationary foraging position within a five body length radius of the focal point. This criterion was chosen because preliminary observations indicated that brook charr rarely interacted with individuals farther away than five body lengths. A continuous commentary of the position of neighbours was recorded because their position often changed during an observation. Population density ($No \cdot m^{-2}$) was estimated as the average number of fish in a 1-m^2 area around the focal fish during the observation.

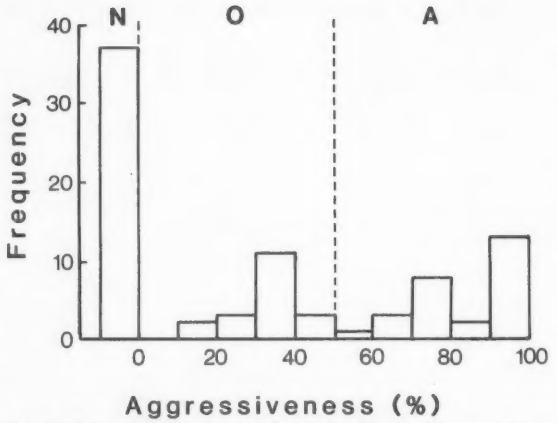

FIG. 1. Frequency histogram of the aggressiveness (percent of conspecific intruders eliciting a charge, chase, nip or approach) of 83 young-of-the-year brook charr. Also shown are the three categories of aggressiveness used in later analyses: N = nonaggressive, O = occasionally-aggressive, A = aggressive.

The fork length of each focal fish was estimated to the nearest 2.5 mm for fish less than 30 mm in length and to the nearest 5 mm for larger fish. The accuracy of these estimates was tested by capturing and measuring a sample of fish after first estimating their length. Estimated length was not significantly different from actual length (paired $t = 0.98$, $n = 38$, $P = 0.33$) and the average difference was 5.3%. Immediately after the observation, I used small aquarium dipnets to catch the focal fish; approximately 50% of the fish were captured. Fish larger than 30 mm were too wary and fast to be easily captured.

To estimate the quality of foraging sites, I returned after 15 min and at the end of the sampling day, from one to several hours later, to see whether the foraging sites of 36 captured fish were reoccupied or not. A site was considered reoccupied if a conspecific had adopted a stationary foraging position within one body length of the foraging site of the former resident. Because of the small sample size, a more liberal classification scheme was used for the removal experiment. Any site that was overtly defended (see above) before capture was classified as defended; all others were classified as not defended. Hence, defence and frequency of intrusion were potentially correlated in this experiment.

Current velocity was measured in the foraging zone of the focal fish at the lesser of one body length or 5 cm above the substrate. I used a Pigmy Teledyne-Gurley meter in 1984–85 and a Marsh McBirney meter in 1986; the two meters gave comparable results (Grant and Noakes 1988).

Captured fish were stored in 10% formalin. Prey items in the oesophagus and stomach were identified and counted under the low power (12.5 ×) of a Leitz Wetzlar dissecting microscope. I counted only intact items to ensure that the stomach contents reflected daytime feeding and were thus comparable with feeding rate data.

Results

Aggressive Behaviour

The frequency of aggressive acts by focal fish was low. Fish of 20–29 mm in length engaged in an average of only 2.0

TABLE 1. Paired comparison of the mean body length (mm) of brook charr of different aggressive categories observed on the same day in the same stream. Each datum was the difference between the means on a single day.

Comparison	Mean length (No. fish)	SE of difference (No. days)	P-value[a]
Aggressive Occasionally-aggressive	44.2 (35) 38.6 (24)	2.2 (17)	0.019
Aggressive Nonaggressive	38.8 (48) 34.4 (50)	1.2 (25)	0.002
Occasionally-aggressive Nonaggressive	37.1 (27) 35.1 (41)	1.5 (20)	0.213

[a]Paired t-test.

aggressive acts per 15 min (range = 0–23, $n = 174$) and no aggressive acts were observed in 60% of the observations lasting at least 5 min. The low frequency of aggression was partly due to a low intrusion rate by conspecifics; only 83 of 174 focal fish were approached by at least three conspecifics. These 83 fish seemed to separate into three groups (Fig. 1), which corresponded roughly to the categories of nonaggressive, occasionally-aggressive, and aggressive.

Aggressiveness and Relative Body Size

Mean body length (mm) of young-of-the-year charr in the three streams increased linearly with day of year: fork length = 0.31 (day of year) − 1.84 ($r^2 = 0.90$, $P < 0.001$; Grant 1987). However, growth rate differed among streams and among years. Therefore, the most powerful test of the relationship between aggressiveness and body length involved a paired comparison of the mean length of fish of different aggressive categories that were observed on the same day and in the same stream (Table 1). Sample sizes differ for each comparison because fish from each category were not observed on all days; for the same reason, means within a category differ slightly between comparisons. Aggressive fish were larger than occasionally-aggressive and nonaggressive fish (Table 1). Occasionally-aggressive fish were larger than nonaggressive fish, but the difference was not significant.

Aggressiveness and Foraging Site Quality

I assumed sites that were reoccupied after the focal fish was removed were of higher quality than sites that were not reoccupied. Of the 20 sites that were defended, 13 were reoccupied within 15 min, whereas only three of 16 undefended sites were reoccupied ($G = 7.79$, df = 1, $P < 0.01$, Sokal and Rohlf 1981). However, the local population density (measured prior to capture) around sites that were reoccupied was higher than around sites that were not reoccupied (9.1 versus 6.1 m^{-2}, $t = 2.50$, $P = 0.018$), so the positive association between defence and reoccupation was potentially confounded by intruder pressure. This possibility seems unlikely because at the end of the day, 12 of 20 defended sites were still reoccupied whereas ony four of 16 undefended sites were reoccupied ($G = 4.36$, $P < 0.05$), and there was no difference in local population densities prior to capture (7.33 versus 7.32 m^{-2}, $t = 0.01$, $P = 0.99$).

Current velocity in the foraging zone of sites that were reoccupied within 15 min was higher than in sites that were not reoccupied (6.5 versus 4.3 $cm \cdot s^{-1}$, $t = 2.99$, $P = 0.005$). The mean current velocity of the reoccupied sites was also not significantly different from 6 $cm \cdot s^{-1}$ ($t = 1.21$, $P > 0.2$), the velocity that Grant and Noakes (1988) suggested was optimal for 20–29 mm fish, whereas the mean current velocity of sites that were not reoccupied was less than 6 $cm \cdot s^{-1}$ ($t = 2.98$, $P < 0.01$). In addition, the foraging rate of captured fish was higher on sites that were reoccupied than sites that were not reoccupied (4.5 versus 3.3 min^{-1}, $t = 2.11$, $P = 0.043$). Therefore, the removal experiment suggested that (1) defended sites were more likely to be reoccupied than undefended sites, and (2) reoccupied sites were of higher quality than sites that were not reoccupied.

Mean lateral foraging radius was used to estimate the size of the foraging area. The effects of aggressiveness on lateral foraging radius was investigated using analysis of covariance with body length and population density as covariates. Population density had no significant effect on lateral foraging radius ($F = 2.80$, df = 1, 166, $P = 0.096$), so it was dropped from the ANCOVA. Larger individuals had larger foraging radii ($F = 204.6$, df = 1, 168, $P < 0.0001$), and the slope of the foraging radius − fork length relationship did not differ significantly among the categories of aggressiveness (ANCOVA, $F = 1.99$, df = 3, 168, $P = 0.118$; Fig. 2). However, the adjusted mean foraging radius increased significantly with increasing aggressiveness ($F = 12.12$, df = 2, 172, $P < 0.0001$; Fig. 2); the mean foraging radius for a 36-mm fish was 44, 38, and 34 mm, respectively, for aggressive, occasionally-aggressive, and nonaggressive fish.

Distance between the focal fish and upstream or lateral neighbours increased with increasing body size of the focal fish ($F = 114.4$, df = 1, 134, $P < 0.0001$) and decreased with increasing population density ($F = 51.6$, df = 1, 134, $P < 0.0001$, ANCOVA). When the effects of body length and population density were controlled for in an ANCOVA, the mean distance to nearest neighbours increased with increasing aggressiveness ($F = 17.1$, df = 2, 134, $P < 0.0001$). The adjusted mean distance to neighbours for aggressive, occasionally-aggressive, and nonaggressive fish of 37 mm in length at a population density of 7.7 $\cdot m^{-2}$ was 142, 115, and 98 mm, respectively. Distance to neighbours and population density were both measures of charr density, but at different spatial scales.

Aggressiveness and Foraging Rate

To relate feeding rate to aggressiveness, I had to control first for body size, because foraging rate and body size are either inversely related (Bachman 1984) or are inversely related when the effects of current velocity are controlled for (J. W. A. Grant, unpubl. data). Although I could not quantify the actual ingestion rate of fish, feeding attempt rate is a reasonable measure of feeding rate because feeding attempt rate correlated positively with number of prey items in stomachs (Spearman's $r = 0.373$, $n = 71$, $P = 0.001$).

There were sufficient data to compare feeding rate for different categories of aggressiveness for two size classes of fish; more-aggressive fish had higher feeding rates for both size classes (Table 2). For 20–29 mm fish, this result may have occurred because more-aggressive fish were found in faster current velocities and because feeding rate increased with current velocity (Grant and Noakes 1988). To control for the effect of current velocity, I compared the mean feeding rate of aggressive and nonaggressive fish in each of nine velocities for 20–29 mm fish: 0, 0.5, 1, 2, 3, 4, 5, 6, and 7 $cm \cdot s^{-1}$. ANCOVA could

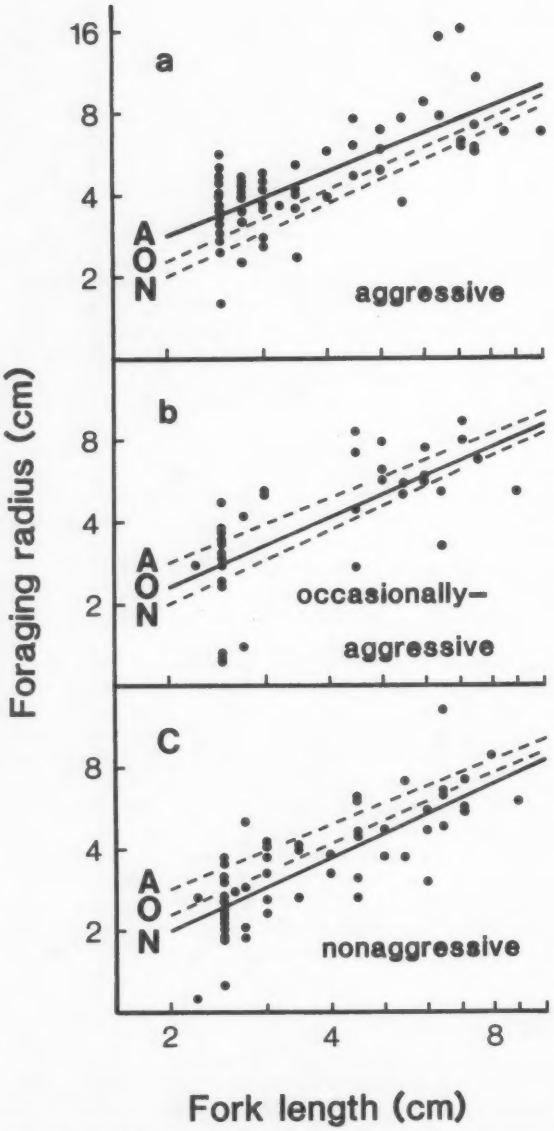

FIG. 2. Lateral foraging radius in relation to body length for (a) aggressive (b) occasionally-aggressive, and (c) nonaggressive brook charr. The solid line represents the least squares regression for the data in each panel; for comparison, the dotted lines represent the regressions for the other two categories of fish (A = aggressive, O = occasionally-aggressive, N = nonaggressive). The regression equations are: A, $\log_{10} Y = 0.79 \log_{10} X + 0.21$, $n = 77$, $r^2 = 0.58$; O, $\log_{10} Y = 0.86 \log_{10} X + 0.10$, $n = 40$, $r^2 = 0.49$; N, $\log_{10} Y = 0.90 \log_{10} X + 0.03$, $n = 59$, $r^2 = 0.60$.

not be used because feeding rate did not increase linearly with current velocity (Grant and Noakes 1988). Twenty-six aggressive and 23 nonaggressive fish were included in the comparison. Aggressive fish had a higher feeding rate than nonaggressive fish independent of current velocity (mean difference = 1.06 feeding attempts · min^{-1}; paired $t = 3.08$, $P = 0.015$). For 40–99 mm fish, the higher feeding rate of aggressive fish (Table 2) was also independent of current velocity, because there was no relation between aggressiveness and mean current velocity for these fish (Grant and Noakes 1988).

TABLE 2. Feeding rate and aggressiveness of brook charr.

Size-class	Parameter	Aggressiveness[a] A	O	N	*P*-value[b]
Feeding attempt rate (No. min^{-1})					
20–29 mm	Mean	3.70	3.04	2.18	0.002
	SD (*n*)	1.62 (37)	2.01 (19)	1.43 (26)	
40–99 mm	Mean	3.88	3.41	2.51	0.021
	SD (*n*)	1.76 (20)	2.54 (18)	1.22 (23)	
Number of prey in stomach					
20–29 mm	Mean	11.1	6.0	5.8	0.118
	SD (*n*)	11.0 (16)	4.1 (10)	6.5 (12)	

[a]A = aggressive, O = occasionally-aggressive, N = nonaggressive.
[b]Kruskal-Wallis test.

The number of prey items found in stomachs increased with increasing aggressiveness, but not significantly ($P = 0.12$, Table 2).

Discussion

More-aggressive individuals were larger than less-aggressive individuals observed on the same day in the same stream. Puckett and Dill (1985) noted a similar trend in coho salmon. There are at least two possible explanations of the relationship: (1) more-aggressive fish acquire better foraging sites and hence grow faster than less-aggressive fish; or (2) larger fish adopt more-aggressive behaviour than smaller fish. Ferguson and Danzmann (1985) suggested a mechanism for the second explanation: rainbow trout (*Oncorhynchus mykiss* formerly *Salmo gairdneri*) with faster developmental rates emerge from the gravel earlier and hence are larger and more-aggressive than fish with slower developmental rates. Mason and Chapman (1965) noted the same trend in coho salmon.

Grant and Noakes (1988) showed that aggressive fish used foraging sites with either faster or less variable current velocities than nonaggressive fish and inferred that aggressive fish occupied the better sites. If reoccupation is an index of preferred foraging sites, then the removal experiment provided more direct evidence that defended sites were of better quality than undefended sites. Much of this "quality" appears to be explained by current velocity and hence renewal rate of drift in the foraging zone.

More-aggressive fish had wider foraging zones and were apparently able to keep neighbours farther away than less-aggressive fish, independent of population density. Even within fish classified as aggressive, those with wider territories had wider foraging zones and were able to keep neighbours farther away (Grant et al. 1989). Presumably, the same correlation explains the differences among the categories of fish in Fig. 2. However, what prevents a nonaggressive fish from exploiting a large foraging zone, but not defending it? Such a fish might attract the attention of neighbouring aggressive individuals and risk being displaced from its foraging site. For example, in coho salmon floaters have higher energetic costs than territorial fish, because they spend time and energy fleeing from territorial fish (Puckett and Dill 1985). Therefore, the optimal size of a for-

aging zone for a nonaggressive fish might be smaller than for an aggressive fish.

There was a positive relation between aggressiveness and feeding rate in brook charr even after I controlled for water velocity, an important component of foraging site quality. I suggest this higher feeding rate, independent of current velocity, was a result of the larger foraging zone and greater distance to neighbours experienced by aggressive fish. By keeping neighbours farther away, aggressive fish presumably decrease competition for potential prey and increase their feeding rate. In contrast, Jenkins (1969) and Bachman (1984) found no relation between dominance rank and feeding rate of adult brown trout. It is possible that their conclusions may be confounded by the effect of body size, because larger fish were of higher dominance rank but fed at a lower rate than smaller fish (Bachman 1984). Unfortunately, the relationship between dominance and feeding rate independent of body size was not examined. McNicol et al. (1985) suggested that aggressive fish fed at a lower rate than nonaggressive fish, although the difference was not significant. However, they classified fish only on the occurrence of chases without taking intrusion rate into account. Therefore, their results may reflect the effects of frequency of aggression on feeding rate rather than effect of aggressiveness.

The positive relation between aggressiveness and feeding rate was not accompanied by a significantly higher number of prey items in the stomachs of more-aggressive fish. The lack of a significant difference may have been due to small sample sizes or simply that most feeding attempts do not lead to ingestion of prey.

The foraging benefits of aggressiveness are summarized below. Aggressive fish were larger, occupied better foraging sites, exploited a larger foraging zone, kept neighbours farther away, and fed at a higher rate than nonaggressive fish. My results are, therefore, consistent with the hypothesis that more-aggressive fish have a higher gross gain rate than less-aggressive fish. This study complements that of Puckett and Dill (1985) who showed that the cost per feeding attempt was lower for territorial fish than for floaters or nonterritorial fish. If their results generalize to brook charr, the net benefits of aggressiveness would be even higher. The crucial study that is required should measure net benefits (i.e. growth rate) of various aggressive tactics in a single population.

Variable	Percent increase of aggressive versus nonaggressive charr
Relative body size	13
Probability that sites are reoccupied	312
Foraging radius	29
Distance to neighbours	45
Feeding attempt rate 20–29 mm fish	70
40–99 mm fish	55
Number prey in stomachs	91[a]

[a]Increase not significant ($P = 0.12$).

If territoriality is subsequently shown to be the best foraging tactic, this does not necessarily mean that all individuals should adopt the defence of foraging sites. The cost of defence for a small fish or for a fish with a poor foraging site might exceed any extra benefit achieved by defence. Fish that defend against smaller intruders only or ignore conspecifics entirely may be "making the best of a bad job" (Dawkins 1980). Such conditional strategies (sensu Dominey 1984) probably explain much of the individual variation in aggressiveness depicted in Fig. 1.

The removal experiment suggested that aggressive brook charr were limiting the access of less-aggressive fish to high quality foraging sites. Therefore, at least at a very small spatial scale territoriality appeared to limit population density. However, to demonstrate that territoriality limits population size, one would also have to show that all nonterritorial individuals die or do very poorly if they do not acquire a territory (see Smith and Arcese 1986). This seems unlikely for 20–29-mm brook charr in these streams, because space did not appear to be limiting (Grant and Kramer 1990).

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Reproductive Strategy in Yellow Perch (*Perca flavescens*): Effects of Diet Ontogeny, Mortality, and Survival Costs[1]

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Hayes, D. B., and W. W. Taylor. 1990. Reproductive strategy in yellow perch (*Perca flavescens*): effects of diet ontogeny, mortality, and survival costs. Can. J. Fish. Aquat. Sci. 47: 921–927.

Factors influencing the theoretical optimal age at first reproduction for yellow perch (*Perca flavescens*) were explored for three different ontogenies including: zooplankton only; zooplankton and benthos; zooplankton, benthos, and fish. Under each of these diets, optimal age at first reproduction decreased as the decrease in mortality caused by reproduction increased. When adult yellow perch fed only on zooplankton, adult survival rate has little effect on optimal age at first reproduction. When fish and benthos are utilized, the optimal age at first reproduction varies strongly with adult survival. Growth rates were affected by both age at maturity and diet ontogeny, with diet ontogeny having a much greater effect. Maximal adult size was affected by reproductive strategy in each case, with larger terminal sizes obtained when maturity was delayed. Increases in size with delayed maturity were greatest when zooplankton, benthos, and fish were available as prey items.

Les facteurs ayant une influence sur l'âge optimum théorique à la première reproduction de la perchaude (*Perca flavescens*) ont été étudiés pour trois régimes différents : zooplancton, zooplancton et benthos, et zooplancton, benthos et poisson. Pour chacun de ces régimes, l'âge optimum à la première reproduction a diminué à mesure que la mortalité causée par la reproduction a augmenté. Dans les cas où la perchaude adulte ne se nourrit que de zooplancton, le taux de survie adulte a peu d'effet sur l'âge optimum à la première reproduction. Lorsque le régime comprend du poisson et du benthos, l'âge optimum à la première reproduction varie fortement avec la survie adulte. Les taux de croissance ont été modifiés par l'âge à maturité et l'alimentation, ce dernier facteur ayant un effet beaucoup plus important. La taille adulte maximale a été modifiée par la stratégie reproductrice dans chacun des cas, les tailles les plus importantes étant atteintes lorsque la maturité était retardée. Les augmentations de taille pour une maturité retardée étaient plus importantes dans les cas où les proies disponibles se composaient de zooplancton, de benthos et de poisson.

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Fishery biologists have long been concerned with age at first reproduction, fecundity, relative gonad weight, and other characteristics of fish stocks that have an impact on their population dynamics, but have made only limited use of theoretical life history models to synthesize the variation in these population characteristics (Schaffer and Elson 1975; Leggett and Carscadden 1978; Glebe and Leggett 1981; Jonsson and Hindar 1982). Most of the emphasis has been on migratory species, possibly because of the strong effect migrations can have on fish life history (Schaffer and Elson 1975; Northcote 1978; Glebe and Leggett 1981). Analyses of the life history of freshwater fishes making very short to no spawning migration have been limited (Jonsson and Hindar 1982).

The goal of this paper is to present a framework for understanding the variation seen in the life history strategy of a nonmigratory fish, yellow perch (*Perca flavescens*), in relation to mortality rates and diet ontogeny. To illustrate the general nature of the relationships betwen these variables, we will apply this model to data collected on slow growing yellow perch (Hayes 1988) from Douglas Lake, Otsego County, Michigan supplemented by literature data where necessary. We chose yellow perch because this species exhibits large inter-population differences in diet ontogeny, mortality rate, and reproductive strategy (Schneider 1972; Thorpe 1977). A general pattern of diet ontogeny has been observed in yellow perch (Schneider 1972). Yellow perch initiate exogenous feeding on zooplankton (Siefert 1972; Hayes 1988). As young yellow perch grow, they typically switch to feeding on benthic invertebrates. The final segment of the diet ontogeny includes fish as the primary component of their diet. These ontogenetic changes are dependent on the size of the fish, and the size spectrum and abundance of these prey items. Typically, if prey resources are available, yellow perch diets shift from zooplankton to benthos when the young-of-the-year reach 30–35 mm in length (Forney 1971; Weber and Les 1982; Guma'a 1978) and from benthos to fish at a size of 150–200 mm (Elrod et al. 1981; Clady 1974).

Methods

We chose to use the methodology of Keyfitz (1977) whereby each potential choice in life history tactic is associated with an equivalent population projection matrix. The optimal life history is then established by comparing the finite rate of increase (or dominant eigenvalue) associated with each projection matrix. This method has the advantage that simple assumptions about the relationship between reproductive effort and survival can be readily incorporated.

[1]Michigan Agricultural Experiment Station No. 11942.

During the optimization process, we restricted the number of life history tactics available to yellow perch with three assumptions.

(1) Choices among life histories were restricted to the case of iteroparity or repeated spawning (Thorpe 1977; Schneider 1984).
(2) Energy allocated toward gonadal development was constrained by the fish's body size at the time of spawning. Further, the maximum gonad weight possible was assumed to be 25% of the fish plus gonad weight (Schneider 1984; Le Cren 1951).
(3) Gonadal development was an all or nothing phenomenon. Gonad weight was thus essentially restricted to zero and to the maximum allowed by the above assumption.

Given this set of assumptions, the primary variables for optimization are age and size at first reproduction. These variables provide useful indicators of the effects factors such as feeding regime can have on life history (Leggett and Carscadden 1978). Additionally, age at first reproduction can provide a measure for testing the validity of theories on optimal life history strategy.

In performing our optimizations, each possible age at first reproduction was associated with an equivalent projection matrix. The dominant eigenvalue of each matrix was then determined, and the optimal reproductive tactic was the one associated with the projection matrix with the highest eigenvalue.

Forming the Projection Matrices

The first step in forming the projection matrix was to estimate net energetic gain across the size spectrum of fish present. These estimates were made from fish collected with graded mesh gill nets in the spring during the breeding season by determining the amount of energy allocated to somatic growth and gonadal growth for the previous year. Somatic growth was determined from the increment in length derived from scale analysis (Ricker 1975). The change in weight associated with this length increment was then multiplied by the caloric content of somatic tissue as determined using an adiabatic bomb calorimeter. Gonad weight and caloric content were also measured for each fish. In Douglas Lake, the caloric content of somatic tissue averaged 810.28 $cal \cdot g^{-1}$ of wet weight ($n = 10$, SE = 66.5) while gonadal tissue averaged 942.37 $cal \cdot g^{-1}$ of wet weight ($n = 21$, SE = 14.7). The sum of somatic and gonadal energy increments represented the net annual energetic intake.

The regression of net energetic intake against fish size at the beginning of the growth season provided us with a size-specific measure of the energy available for growth and/or gonadal development for the next year. Thus, assuming that net energetic gain not used for gonadal development is available for somatic growth, a fish's somatic weight increment can be estimated under the condition of no gonadal growth or of complete gonadal development (Jones and Johnston 1977).

Energy invested in gonadal development was converted to fecundity using data from fish caught in Douglas Lake where:

$$\#\ \text{eggs} = \text{calories invested}/1.4607\ cal \cdot egg^{-1}$$

A 1:1 sex ratio was used (Thorpe 1977), and the number of eggs was multiplied by 0.5 to represent the female portion of the population on which projection matrices are based (Leslie 1945).

Net caloric gain for young-of-the-year yellow perch averaged 870 cal, with weight at hatch being 0.001 g (Hayes 1988). With these values, fecundity and weight at age were determined from the net energetic intake equation for each matrix representing a particular age at first reproduction. These procedures allowed us to construct the first row for each of the projection matrices. This row contained our calculated age specific fecundities.

Age-specific survival rates in each matrix were composed of two components. The first component was the age-specific survival rate for fish not reproducing (referred to as the basal survival rate). The second component dealt with reproductive age classes, where the basal survival rate was multiplied by a constant factor (theta) which represented the proportional decrease in basal survival rate due to reproduction. Because survival rates vary widely among yellow perch populations, and survival rate can be altered by fishing (Heyerdahl and Smith 1971; Thorpe 1977), the optimal age at first reproduction was determined for a range of adult survival rates representative of those reported in the literature (Thorpe 1977). Because the values of theta have never been precisely measured, we approached the problem of determining the effects that different theta values have on the optimal life history solution (age at first reproduction) using sensitivity analysis. The only study we are aware of that provides an indication of the proportional decrease in survival due to reproduction for yellow perch is Schneider (1972). In this study, he observed that, in a lake containing only yellow perch, annual survival of immature 1-yr-old perch was typically 75%. For perch greater than 2-yr-old, which were reproductive, the annual survival rate was typically 59%. The proportional decrease in survival due to reproduction (theta) was 0.79. Thus, we used 0.75 as a lower bound of theta. As an upper bound to theta, we used 1.0 as this is the value at which reproduction does not decrease survival rate at all.

Determination of Optimal Age at Maturity

Matrices were formed representing initial maturity at age 1–6. The dominant eigenvalue (lambda) of each of these six matrices was determined using the power method described by Keyfitz (1977) and Johnson and Riess (1981). The matrix having the largest dominant eigenvalue is the optimum among the set being considered. All life histories were compared for lambda approximately equal to 1 which is the value representative of a stationary population (Pielou 1974). When lambda >1, earlier maturity is favored; when lambda <1, later maturity is favored (Stearns 1976). Thus, for general comparisons, a stationary population provides a common basis.

Results

Case I: Zooplankton Diet

When perch are feeding solely on zooplankton, the net energetic gain of postyearling perch decreases linearly with body weight (Fig. 1). This is the case for the majority of the yellow perch population in Douglas Lake (Hayes 1988). The regression equation describing these data ($n = 58$; $p < 0.05$) is:

$$E = 12047 - 436W \qquad R^2 = 0.29$$

where,

E = Net annual energetic gain in cal.
W = Weight in g at beginning of a year.

The negative slope of the regression line in the zooplankton only diet indicates that, as fish get larger, the energy available

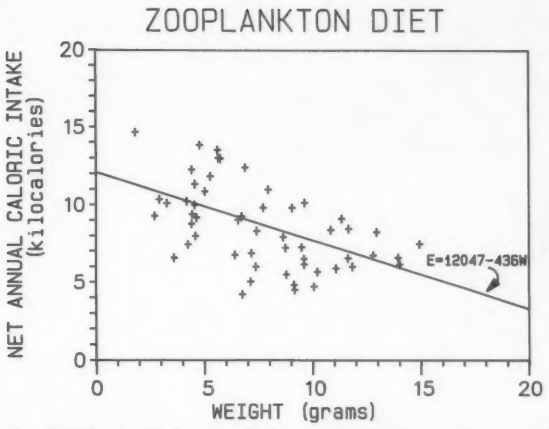

FIG. 1. Relationship between body weight at beginning of year, and net energetic intake during that year for case of feeding on zooplankton only.

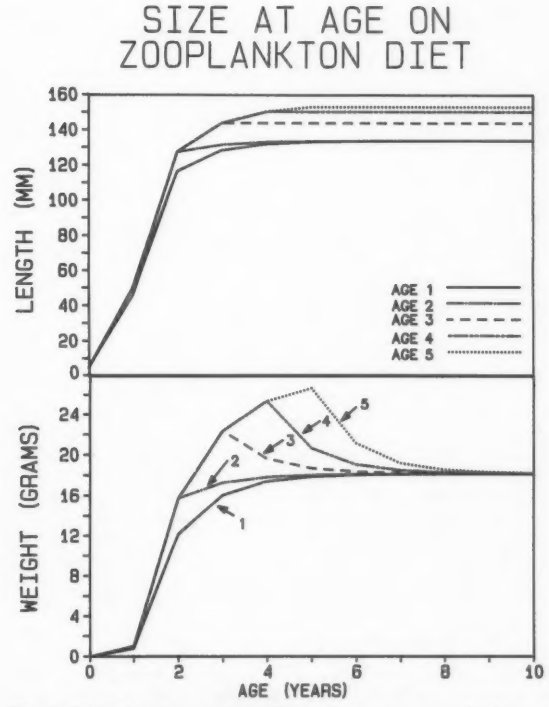

FIG. 2. Body size at age under different first year at reproduction tactics for case of zooplankton only diet.

for somatic growth and reproduction is increasingly limited. We postulate that this occurs because larger fish have higher total metabolic costs (Ware 1975) that cannot be offset solely by increased zooplankton consumption (Werner 1974).

In this case, progressively greater maximal lengths are attained as reproduction is delayed (ages 1–6) with length and weight both reaching an asymptote by ages 5 and 8, respectively (Fig. 2). The asymptotic nature of the length and weight at age relationships is a function of the negative slope of the energy

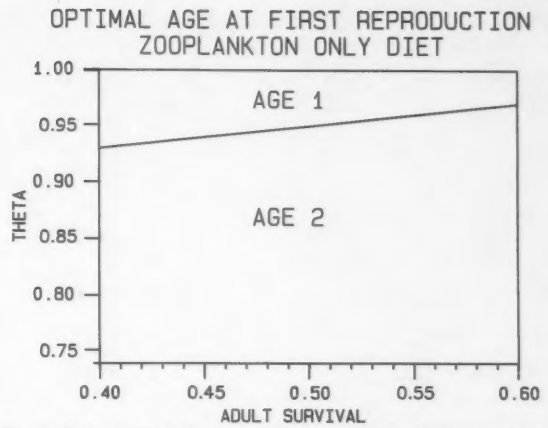

FIG. 3. Optimal age at first reproduction as a function of adult survival rate and effect of reproduction on survival (theta).

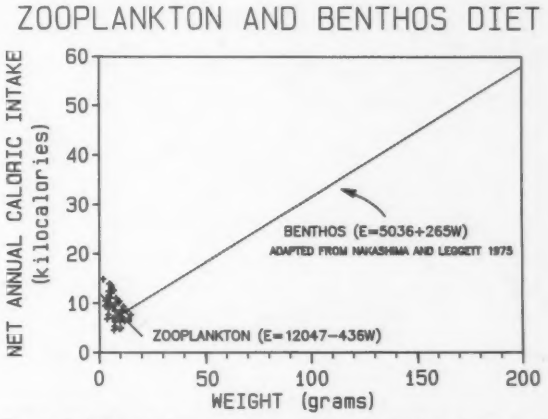

FIG. 4. Relationship between body weight at beginning of year, and net energetic intake during that year for case of feeding on zooplankton and benthos. The benthos feeding line was adapted from Nakashima and Legett (1975).

intake line. The negative slope of the energy intake line implies that at some size energy intake equals reproductive allocation, leaving no residual energy for somatic growth. In fact, if reproduction is delayed to age 3 or later, energy derived from a zooplankton diet is insufficient to meet the energy demands for full gonadal development. In order to satisfy these energetic demands, energy is diverted from somatic tissue to gonadal development. Thus, perch somatic weight at age markedly declines among fish that mature at age 3 or later (Fig. 2).

Within the range of adult survival rates (0.4–0.6) and reproductive effects (thetas = 0.75 − 1.00) in our sensitivity analyses, the optimal age at first reproduction was between age 1 and age 2 (Fig. 3). Under this scenario, adult survival rate had little effect on optimal age at first reproduction, whereas the value of theta is critical in determining which age is optimal.

Case II: Zooplankton and Benthos Diet

Very few yellow perch in Douglas Lake fed on benthic invertebrates (Hayes 1988). Thus, to adequately model the effects of an ontogenetic shift in diet from zooplankton to benthos on

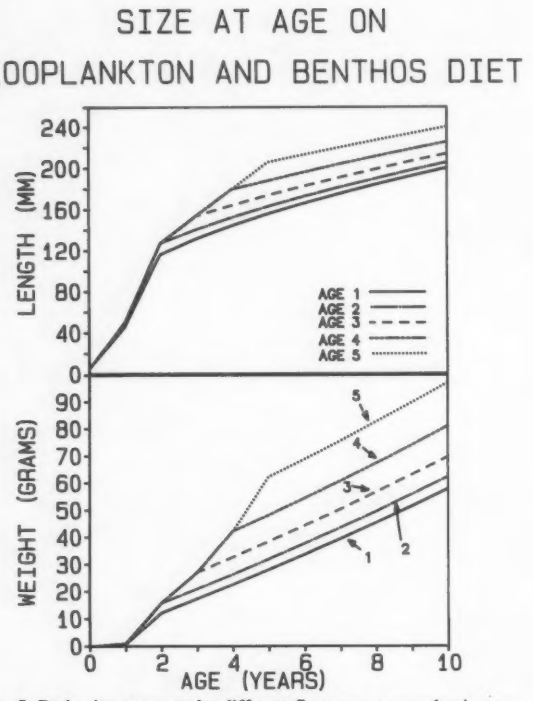

FIG. 5. Body size at age under different first year at reproduction tactics for case of zooplankton and benthos diet.

reproductive strategy, we adapted data from Nakashima and Leggett (1975) for perch in Lake Memphremagog (Quebec-Vermont). To utilize their data, we used the energetic equivalents from Douglas Lake to convert their weight at age data to energy increments. We used the slope of the regression results from their data to model the effects of fish weight on energy intake (Fig. 4). Additionally, the intercept was adjusted to intersect with the energetic intake line for feeding on zooplankton only (case 1) for evaluation of the energetic consequences of shifting diets from zooplankton to benthos. This line has a positive slope because, as perch become progressively larger, they are able to handle and consume progressively larger benthic invertebrates (Werner 1974). Perch <10 g were considered to be planktivorous, while perch >10 g were considered to be benthivorous. The energetic intake equations used to model the two segments of this diet ontogeny were:

$$E = 12047 - 436W \text{ for } W<10 \text{ g Zooplankton segment}$$
$$E = 5036 + 265W \text{ for } W>10 \text{ g Benthos segment}$$

When benthic invertebrates are added to the diet ontogeny of perch, the maximum size attained by these fish is substantially greater than those feeding only on zooplankton, regardless of the age at first reproduction. Additionally, length and weight at age were not asymptotic, and the growth trajectories diverge between each reproductive tactic at an early age (Fig. 5).

Unlike the case where perch fed only on zooplankton, the optimal age at first reproduction varied with both adult survival and theta (Fig. 6). Optimal ages for maturity increased as adult survival increased and theta decreased. Changes in the optimal

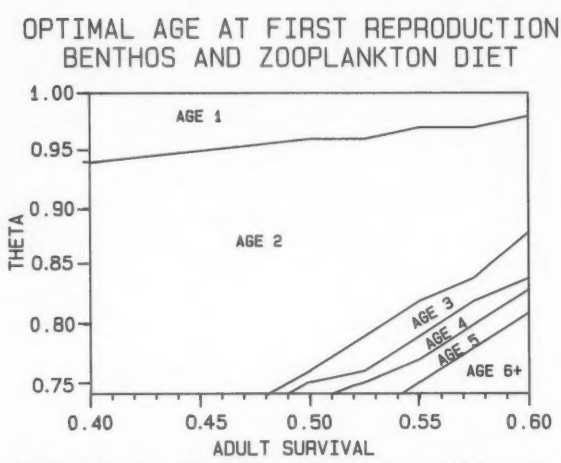

FIG. 6. Optimal age at first reproduction as a function of adult survival rate and effect of reproduction on survival (theta).

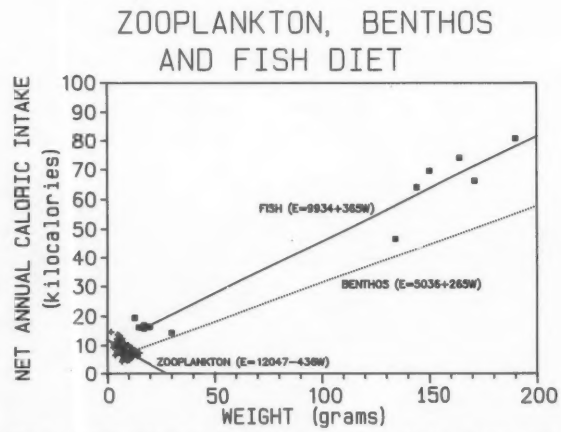

FIG. 7. Relationship between body weight at beginning of year, and net energetic intake during that year for case of feeding on zooplankton, benthos and fish. The benthos feeding line was adapted from Nakashima and Leggett (1975).

age at maturity were most sensitive with adult survival rates higher than 0.50 and values of theta less than 0.89.

Case III: Zooplankton, Benthos, and Fish Diet

Perch in Douglas Lake >30 g were observed to have a greater proportion of fish and benthos in their diet than their smaller counterparts, with the proportion of fish in the diet increasing with size (Hayes 1988). The regression of energetic intake versus weight (Fig. 7) for these size perch in Douglas Lake was:

$$E = 9934 + 359W \quad R^2 = 0.96 \quad n = 12$$

Thus the set of energetic equations used to model this diet ontogeny were:

$$E = 12047 - 436W \text{ for } W<10 \text{ g} \quad \text{Zooplankton segment}$$
$$E = 5036 + 265W \text{ for } W>10 \text{ g and } W<30 \text{ g} \quad \text{Benthos segment}$$

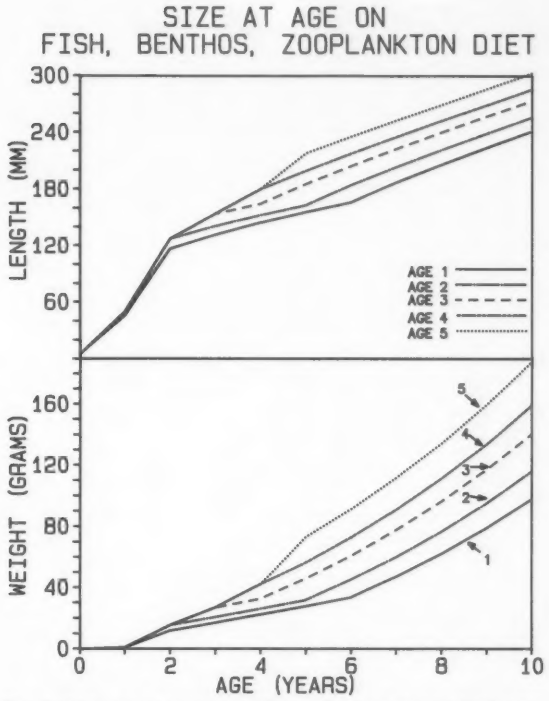

FIG. 8. Body size at age under different first year at reproduction tactics for case of zooplankton, benthos and fish diet.

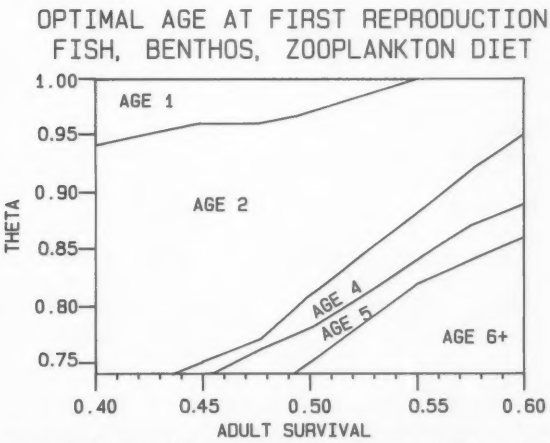

FIG. 9. Optimal age at first reproduction as a function of adult survival rate and effect of reproduction on survival (theta).

$$E = 9934 + 359W \text{ for } W > 30 \text{ g} \qquad \text{Fish segment}$$

Under this feeding regime, size at age increases under all ages at first reproduction, with the growth trajectories associated with each reproductive tactic diverging rapidly after age 4 (Fig. 8). Thus, age at first maturity has a larger impact on growth in this case than in the previous two cases.

As in case II where perch fed on zooplankton and benthos, optimal age at first reproduction was sensitive to and varied widely with both adult survival rate and theta. However, the optimal age at maturity for a specific combination of adult survival and theta is generally higher than for yellow perch feeding on benthos. For example, if adult survival is 0.55 and theta is 0.80, the optimal age at reproduction is age 6 when fish are included as a diet item (Fig. 9), but the optimal age drops to age 3 when the diet ontogeny includes only zooplankton and benthos (Fig. 6).

A result that is specific to the fish feeding energetic equations in this case is that under no conditions was age 3 the optimal age at first reproduction. This is due to the discontinuity between the benthos feeding line and the fish feeding line (Fig. 7) which probably reflects our lack of field data on these transition zones. If the transition between these energetic intake lines was smooth, it is likely that age 3 would be included as an optimal age at first reproduction for some combinations of adult survival and theta.

Discussion

The optimal age at first reproduction for yellow perch is affected simultaneously by theta, adult survival rate, and by the size-specific net energetic relationship as determined by diet composition. However, optimal age at reproduction is relatively insensitive to changes in the value of these parameters over certain ranges. For example, when perch are feeding on zooplankton only and have energetic intake as defined in case I, optimal age at reproduction is insensitive to changes in adult survival and theta over a broad range of values. However, a relatively small change in adult survival can produce a change in optimal age at reproduction from age 6 to age 2 when perch diets include zooplankton, benthos, and fish (see case III).

The sensitivity of age at maturity to changes in survival, theta and energetic intake must be evaluated in terms of the magnitude of variation that are likely to occur. Exploitation by a fishery on perch has been observed to decrease adult survival rates by as much as 45% (Heyerdahl and Smith 1971). Large changes in adult survival such as this can account for substantial changes in age at maturity if the population has good growth rates associated with feeding on benthos or fish. However, if perch are feeding only on zooplankton little or no change in age of maturity would be expected.

Additionally, size-specific energy intake rates can be altered by man's activities, especially where degradation of the environment reduces the productivity of prey populations. An example where such degradation has occurred is in Lake Erie, where abundance of large benthic invertebrates such as *Hexagenia* decreased when dissolved oxygen concentration fell (Hartman 1972). The loss of these prey items has several effects. First, the slope of the net energetic intake line decreases since larger fish have less of an advantage over smaller fish in their ability to handle prey items when all prey items are relatively small. Second, the overall level of the energetic intake line will decrease if the general abundance of a prey type is reduced forming a block in diet ontogeny. The slope and intercept of each segment of the diet ontogeny thus summarize the bioenergetics relationships between different sizes of the predator in question and its prey. The intersection of the different segments of the energetic intake line further provides a means for estimating the size where diet shifts are expected to occur. Assuming fish are maximizing their net energetic intake, fish should switch diets at the size where the energetic intake line

segments intersect. This intersection point will thus change with changes in prey abundance or size structure.

Changes in prey availability may also occur through the introduction or removal of competing species. Changes in the energetic intake line mediated by altered prey availabilities can result in dramatic shifts in age at maturity, as can be seen by comparing zooplankton only diet (Fig. 3) and zooplankton, benthos, and fish diet (Fig. 6). Such changes in the age at maturity can significantly impact growth (Figs. 2, 5, 8) affecting the quality of the fishery for sport and commercial use. As such, management options may have a significant impact on the reproductive biology of perch which in turn affects somatic growth rates.

Our results indicate that diet ontogeny and size-specific energetic intake rates are more significant in determining ultimate fish size than reproductive strategy. This is analogous to Glebe and Leggett's (1981) conclusion that "inter-population differences in energy allocation to migration versus reproduction are a consequence, rather than a cause, of the different life history strategies exhibited by populations of shad over its Atlantic Coast range".

In summary, it appears that energy rich prey such as benthos or fish are necessary for maturity to be delayed beyond age 2 for yellow perch. The availability of such prey, however, is not sufficient cause for delayed reproduction. A combination of relatively high adult survival rate and high mortality due to reproduction (low theta) are also necessary conditions.

Acknowledgments

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Appendix

Formulation of matrices (Leslie 1945) used to determine optimal reproductive strategy.

Body weight over time was computed recursively, with the initial value:

$$W_0 = 0.001 \text{ g}.$$

Energy intake during the first year was 870 cal, which would result in a body weight at age 1 of 1.075 g if the perch did not elaborate gonadal tissue. If the perch did mature in its first year of life, it would reach a weight of 0.833 g.

After this age, the recursive equations used were:

if not mature at age t:

$$W_t = W_{(t-1)} + \frac{W_{(t-1)} \cdot \text{energetic slope} + \text{energetic intercept}}{810.28 \text{ cal} \cdot \text{g}^{-1}}$$

if mature at age t:

$$W_t = W_{(t-1)} + \frac{W_{(t-1)} \cdot \text{energetic slope} + \text{energetic intercept}}{810.28 \text{ cal} \cdot \text{g}^{-1}}$$

$$- \frac{W_{(t-1)} \cdot 0.25}{942.37 \text{ cal} \cdot \text{g}^{-1}} - 0.225259 \cdot W_{(t-1)} \cdot \text{energetic slope}$$

$$+ \text{energetic intercept} - \frac{W_{(t-1)} \cdot 0.25}{942.37 \text{ cal} \cdot \text{g}^{-1}}$$

The energetic slope and energetic intercept are the slope and intercept obtained from the regression of net annual energetic intaken on body weight.

The final two terms in the equation for calculating the body size of mature individuals represent energy losses to gonadal development. The first represents the amount of energy needed to elaborate gonadal tissue equal to 25% of the body weight at the beginning of the year. The second term represents the energy required for developing gonadal tissue equal to 25% of the current year's growth.

Fecundity at age was calculated as:

$F_t = 0$	if not mature at that age
$F_t = 0.25 \cdot W_t \cdot 760.37$	if mature at that age

Survival at age:

S_t = basal survival	if not mature at that age
S_t = basal survival · theta	if mature at that age

Predictors of Relative Fish Biomass in Lakes and Reservoirs of Argentina

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Relationships between total fish biomass and chlorophyll, nutrient levels, and morphometric and climatic factors are shown for a set of 106 Argentinian lakes and reservoirs. The total data base is highly heterogeneous. Relative fish biomass (CPUE) was estimated from gill net catches. A data screening process was applied to the environmental data base to homogenize it. Nutrient, total organic matter content, and mean depth were most important in explaining relative fish biomass variability between lakes and reservoirs. In most heterogeneous sets total organic nitrogen (TON) explained most of CPUE variability. After screening for limnological anomalies (i.e. turbidity derived from inorganic sources, unsuitable conditions for fish, and TN/TP (total nitrogen / total phosphorus) < 37 (molar basis)), TP and TON were highly correlated with CPUE. Mean annual air temperature was significantly related to fish biomass, but the relationship was not significant after nutrients, morphometry, and environmental oxygen conditions had been accounted for. Several possible causes are discussed. The results support the hypothesis that fish/phytoplankton, and fish/zooplankton biomass ratios are inversely related to lake trophy.

Les relations entre la biomasse totale de poissons et la chlorophylle, la teneur en éléments nutritifs, et les facteurs morphométriques et climatiques sont présentées pour une série de 106 lacs et réservoirs argentins. La banque de données totale est très hétérogène. La biomasse de poissons relative (CPUE) a été estimée à partir des prises au filet maillant. Un tri des données a été effectué dans la banque des données environnementales afin de l'uniformiser. Les éléments nutritifs, la teneur totale en matières organiques et la profondeur moyenne ont été très importants pour expliquer la variabilité de la biomasse relative de poissons entre les lacs et les réservoirs. Dans la majorité des séries hétérogènes, la teneur en azote organique total (AOT) expliquait en majeure partie la variabilité de CPUE. Après un tri des anomalies limnologiques, c'est-à-dire : la turbidité dérivée de sources inorganiques et les conditions impropres pour le poisson, et un rapport TN/TP inférieur à 37 (base molaire), le phosphore total et l'azote organique total ont été étroitement corréliés avec CPUE. La température ambiante annuelle moyenne affichait une relation étroite à la biomasse de poissons, mais cette relation n'était pas significative une fois prises en considération la teneur en éléments nutritifs, les caractéristiques morphométriques et la teneur en oxygène de l'environnement. Plusieurs causes possibles sont envisagées. Les résultats obtenus viennent étayer l'hypothèse selon laquelle les ratios de biomasse poisson/phytoplancton et poisson/zooplancton sont inversement reliés aux caractéristiques trophiques du lac.

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Empirical or predictive limnology is fundamental to fish management because it offers biologically and socially important predictions that have been confirmed in repeated trials (Peters 1986). Regression equations for predicting potential fish yields from freshwater lakes and reservoirs have become common in the fisheries literature (Schlesinger and Regier 1982); however, homogeneity of the data base has been of concern for correct use of predictive models (Henderson et al. 1973; Ryder 1982). In regions with many almost unknown water bodies, an ecosystem level, comparative approach (Regier and Henderson 1980), including limnological and fish characterization of lakes and reservoirs, seems to be more efficient than work at fish species population levels (Regier and Henderson 1980). Such an approach also allows testing the applicability of general models. Furthermore, comparative studies can provide transition between empirical management and explanatory theory (Collins and Sprules 1983; Hoenig et al. 1987).

Argentina covers a highly varied geographic area exhibiting marked diversity in climate and geology. There are more than 400 lakes and reservoirs with surface areas over 5 km^2 and only about 20 have been intensively studied (Quiros et al. 1983). Most of the Argentinian lakes and reservoirs are lightly exploited for fish. Only a few very shallow lakes in the Pampa Plain and no more than three lakes in the Patagonian Plateau support a commercial fishery. The remainder of lakes and reservoirs support lightly fished or unevaluated sport fisheries. A few lakes and reservoirs have also nonintensive domestic fisheries.

My working hypothesis was that general external characteristics of an aquatic system determine many of its internal biological features (Brylinsky 1980; Kerr 1982) or that climatic, morphometric, and edaphic factors and their interrelationships determine lake productivity (Rawson 1955). I had expected that this general hypothesis could be useful for ordering Argentinian freshwaters on the basis of fish biomass. Lakes and reservoirs may be grouped by fish species composition (Tonn et al. 1983; Marshall and Ryan 1987) or by environmental factors. Here, the first aim was ordering lakes and reservoirs on the basis of fish biomass; therefore, I focused primarily on abiotic factors and clorophyoll, a measure of potential primary productivity at the lowest trophic level.

Materials and Methods

This paper relates the mid-summer relationships between fish biomass and chlorophyll, nutrient levels, and morphometry and climatic factors for a set of 106 Argentinian lakes and reservoirs (ARLARE data set).[1] During the summers of 1984 to 1987, 110 lakes and reservoirs of Argentina were sampled. Four lakes without fish were excluded. Lakes and reservoirs were visited once, except for six, situated in Patagonia Plateau (Fig. 1), that were sampled seasonally over 2 yr. Reservoirs with low hydraulic residence time (<3 mo), and floodplain lakes and ponds situated in the Plata River Basin were expressly excluded (Bonetto et al. 1969; Quiros and Cuch 1983; Quiros and Cuch 1989).

Data Collection

Lake surface area (A), latitude (*LAT*) and elevation (ALT) were obtained from 1 to 50 000 and 1 to 10 000 topographic maps (Quiros et al. 1983). Bathymetric surveys with a SIMRAD Skipper 411 model echosounder, a hand winch and line and lead (Welch 1948) were conducted on 47 lakes. We obtained mean depth (ZMEAN ± 10%) for each lake and reservoir. For most of the reservoirs, morphometric parameters were taken from topographic maps before impoundment (Quiros et al. 1983). For eight lakes, mean depth was estimated from the regression between mean depth and area for other lakes in the same region. Mean annual air temperature (TEMP) and frost free period (FFP) were obtained from Quiros et al. (1983); these data were considered to be of low quality especially in the Patagonian Andes Region and Tierra del Fuego (Quiros and Drago 1985), because of the sparsity of meteorological stations. Sampling stations were situated over the deepest part of the basin for lakes, and 500 m to 2 km from the impounding dam for reservoirs (Quiros 1988). For each lake, vertical profiles were obtained for temperature, dissolved oxygen, conductivity, pH, and total alkalinity. In lakes < 25 m deep, a Hydrolab Surveyor system was used. Water samples were taken from surface to bottom with a Friedinger plastic sampler (Golterman et al. 1978).

Transparency measurements (SDL) were obtained with a 25-cm black and white Secchi disk. True color (COL) was determined in the field with a Hazen platinum-cobalt scale. Conductivity (20 °C, K20), dissolved oxygen (DO, by the Winkler method) and total alkalinity (TA, by potentiometric titration), were measured in the field. Total dissolved solids (TDS) was determined as total residue on evaporation to constant weight at 105 °C, and water hardness (HARD) was determined volumetrically. Chlorophyll (CHL), total phosphorus (TP), total organic nitrogen (TDN), total organic carbon (TOC), nitrate, nitrite, major ions, and total dissolved solids (TDS) were determined from samples collected at the 0.5-m depth. Analytical methods used have been described previously (Quiros 1988).

Chlorophyll was determined according to Stauffer et al. (1979) without pheopigment corrections. All nutrient and chlorophyll determinations were done in duplicate. For 71 lakes and reservoirs in northern Patagonia, and central-western and northwestern arid region, nitrate and nitrite were determined with an ORION D793-07 electrode and the diazotation method (APHA et al. 1975), respectively. On average, the nitrate plus nitrite-N fraction was 0.24 of Kjeldahl-N (SE = 0.16, range: 0.03–1.16). For 35 lakes in the Pampa Region, southern Patagonia and Tierra del Fuego, I assumed nitrate plus nitrite was 20% of the total Kjeldahl nitrogen concentration. Nitrate and nitrite were used only to estimate TN:TP ratios.

Fish were collected by nylon gillnets. The experimental net was composed of nine 50-m panels of 42, 51, 60, 70, 78, 105, 120, 140, and 170-mm stretch mesh. In the Pampa Plain lakes, nine panels of 25 m were used. Gillnets were set in gangs of floating nets, perpendicular to the shoreline, with the smallest mesh inshore. The sets were remote from stream mouths, protected bays or islands. Lakes larger than 20 km^2 or with irregular shorelines were fished for 2 d at two locations. Floating sets were made overnight, usually for 12 h. I used only data from the overnight catches. Relative biomass of fish was approximated using mean weight (kg) of fish caught per overnight set for the experimental gang of gillnets (Northcote and Larkin 1956). Catch was standardized as the sum of mean weights of fish in 100 m^2 of each mesh, for the nine panels (CPUE).

Zooplankton samples were collected by 50-m vertical tows or from 0.5 m above the bottom to the surface. In both cases epilimnetic waters were sampled. 100% net efficiency was assumed; Menu Marque and Marinone (1986) detailed enumeration and biomass determination methods. Total zooplankton biomass (TOZOO, μg dry weight$\cdot L^{-1}$) was used herein.

Data Analysis

The data (ARLARE) is highly heterogeneous and the climatic, morphometric, and edaphic variables are closely related (Quiros 1987). All lakes and reservoirs are contained within 24 and 55 °S latitudes (Fig. 1).

Exploratory analyses of the data base were made by simple and partial correlation and simple and stepwise multiple regression analyses (Weisberg 1980; Davis 1986). Number Cruncher Statistical System (NCSS) for IBM PC (Hintze 1985) statistical package was used. For comparison between regression models, ANCOVA analysis and general *F*-testing was used (Weisberg 1980). Environmental variables were log-transformed except LAT, TEMP, and DOb levels. Water type was characterized as differences in anionic (A/A, carbonate plus bicarbonate to chloride plus sulfate) and cationic (C/C, calcium plus magnesium to sodium plus potassium) ratios (equivalent basis). Environmental ordination of lakes and reservoirs was performed by principal components analysis (PCA) (Davis 1986). A correlation matrix analysis was used because variables were in different units. Here I present the results of an environmental PCA ordination of lakes and reservoirs with 19 variables. All the variables except LAT, TEMP, and DOb were log-transformed to linearize the relations and to reduce the disproportionate effect of the untransformed data from a few lakes and reservoirs (Peters 1986). The results of the PCA did not change appreciably if CHL was or was not included in the analysis.

Different pathways of data screening were tried to improve predictive ability. Here we present the pathway that leads to explaining the highest proportion in CPUE variance using the fewest variables. Lakes and reservoirs with limnological anomalies or unsuitahle conditions for fish were removed in a stepwise procedure. Lakes and reservoirs known to be subject to

[1]Complete set of tables is available, at a nominal charge, from the repository of unpublished data CISTI, National Research Council of Canada, Ottawa, Ont. K1A 0S2, or from the Instituto Nacional de Investigacion y Desarrollo Pesquero, C.C. 175, 7600 Playa Grande, Mar del Plata, Argentina.

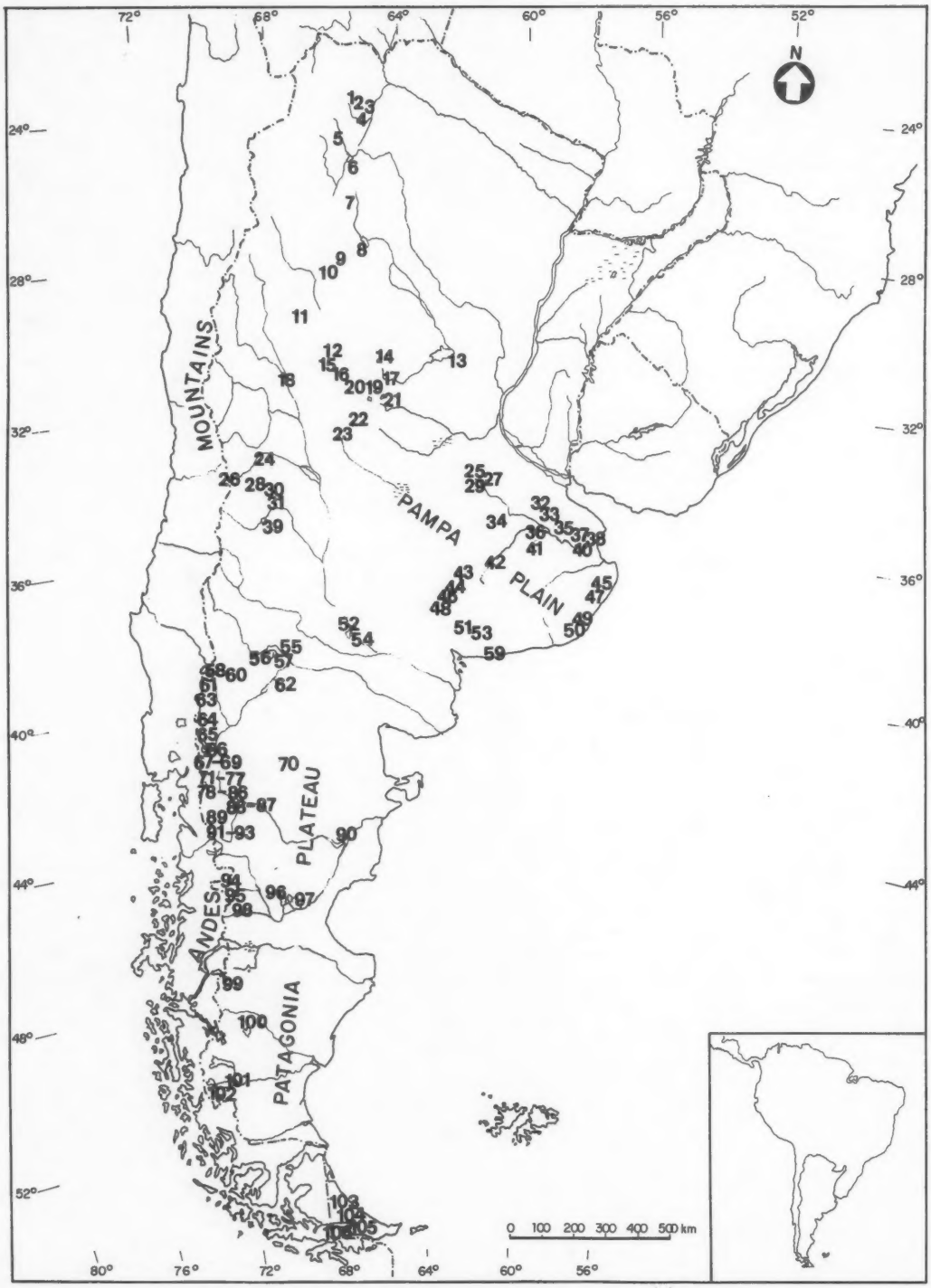

FIG. 1. Argentina showing the location of the 106 lakes and reservoirs studies.

TABLE 1. Mean and range for the limnological and fish characteristics measured in Argentinian lakes and reservoirs (data set #2, $n = 99$). Area (A, km^{-2}), mean depth (ZMEAN, m), mean annual air temperature (TEMP, °C), elevation (ALT, m), total dissolved solids (TDS, $g \cdot m^{-3}$), total phosphorus (TP, $mg \cdot m^3$), total organic nitrogen (TON, μM), total chlorophyll (CHL, $mg \cdot m^{-3}$), carbonate plus bicarbonate to chloride plus sulfate (A/A, equivalent basis), calcium plus magnesium to sodium plus potasium (C/C, equivalent basis), bottom dissolved oxygen concentration at sampling (DOb, $mg \cdot L^{-1}$), catch per unit effort of Atherinidae (CPUEA, kg/night per standard gillnets), total catch per unit effort (CPUE, kg/night per standard gillnets), and number of fish species at sampling (NUM).

	lakes ($n = 70$)		reservoirs ($n = 29$)	
Variable	Mean	Range	Mean	Range
A	80.0	0.09–1466.0	81.6	1.5–816.0
ZMEAN	32.4	0.7–166.0	21.9	3.0–79.0
TEMP	9.7	3.0–16.5	15.9	5.0–20.4
ALT	464	2.0–3250.0	764	169.0–1339.0
TDS	1043	19.0–13800.0	640	33.0–6780.0
TP	131	1.0–1288.0	63	3.0–477.0
TON	104	5.0–762.0	50	6.0–352.0
CHL	26.1	0.16–405.0	17.7	0.66–218.0
A/A	5.56	0.03–26.6	2.47	0.04–9.83
C/C	2.47	0.03–16.0	2.62	0.18–8.05
DOb	8.2	0.5–12.2	4.2	0.01–9.7
CPUEA	34.8	0–435.2	23.5	0–283.0
CPUE	88.2	1.2–505.1	37.3	1.9–283.0
NUM	3.3	1.0–9.0	3.6	1.0–10.0

TABLE 2. Data screening conditions for total lakes and ponds (LA) and reservoir (RE) data sets analyzed. DOb (bottom dissolved oxygen at sampling; $mg \cdot L^{-1}$), CHL:TP (chlorophyll to total phosphorus ratio), TN:TP (total nitrogen to total phosphorus ratio, molar basis), NUM (number of fish species at sampling).

Data set	n	Data screening conditions
#1	106	—
#2	99	—
#2RE	29	Reservoirs
#2LA	70	Lakes
#3	74	CHL:TP>0.05, DOb>2.0 $mg \cdot L^{-1}$
#3LA	47	Lakes, CHL:TP>0.05, DOb>2.0 $mg \cdot L^{-1}$, TN:TP>37
#4LA	37	Lakes, CHL:TP>0.05, DOb>2.0 $mg \cdot L^{-1}$, TN:TP>37, NUM>1

extensive human influence were excluded (set #2, Table 1). The data screening process and the lakes and reservoirs used in this analysis are in Table 2.

In many lakes and reservoirs in Patagonian Andes, Tierra del Fuego, and the central-western and northwestern regions, fish components have not coevolved with each other and with their environment, due to fish introductions and fish transfers. As a result, I used water temperature and dissolved oxygen levels at sampling, number of fish species (NUM) in the catch and frequency of Atherinidae (%ATE) as surrogate variables.

Flushing rate modifies thermal stratification of reservoirs in general (Wunderlich and Elder 1967; Wunderlich 1971) and Argentinian reservoirs in particular (Viana and Quiros 1989). Furthermore, flushing rate is related to nutrient loading. Therefore, differences in fish biomass may be attributable in part to flushing rate (Jenkins 1982). The relationship between fish biomass and mean hydraulic residence time (Tw) was examined here for a subset of 43 lakes and reservoirs, for which flushing rates were known.

Results

Lake Characteristics by Region

Central western and northwestern arid region

Throughout the arid region 29 lakes and reservoirs were examined, 25 of which were reservoirs. Mar Chiquita Lake and El Cadillal Reservoir were removed from our set #2 analysis. The former is a saline lake with very high total dissolved solids (28 157 $mg \cdot L^{-1}$). The latter has been developed for live-stock, has high relief, significant land erosion and therefore, high reservoir inorganic turbidity. The high-mountain lake (El Diamante) was used within the Patagonian data set.

TEMP ranged from 11 to 16 °C for the central western to 16 to 20.4 °C for the northwestern lakes and reservoirs. There were 12 reservoirs and two lakes (most ($n = 13$) in the northwest) with DOb below 3.0 $mg \cdot L^{-1}$. CPUE ranged from 1.9 to 283.0 kg/night.

Most of the fish species of the reservoirs have been introduced (Baigun and Quiros 1985; Quiros 1987) from outside Argentina or from other river basins in Argentina. Salmonid introductions were attempted but without much success (Baigun and Quiros 1985; Quiros 1987). Argentinian silverside (*Basilichthys bonariensis*, Atherinidae), introduced from the Pampa Plain region, was the most common (frequency of occurrence: 0.49). Rainbow trout (*Oncorhynchus mykiss*), reintroduced principally from southern Patagonia, had a low mean frequency of occurrence (0.2) and was captured only in the western reservoirs and in one northern lake at an elevation of 1446 m. One species of Percichthyidae had been introduced from Patagonia and was captured in the western reservoirs and in one northern lake (mean frequency of occurrence: 0.13). Remaining species were carp (*Cyprinus carpio*), or species native to their respective catchment basins (Quiros et al. 1988). More than 80% of the fish biomass arose from exotic species in the central-western and northwestern arid region.

Patagonia and Tierra del Fuego

Fifty lakes and reservoirs were sampled throughout Patagonia and Tierra del Fuego; most ($n = 45$) were lakes. Ne-Luan, Cronometro, Blanco, and Escondido lakes were included only in the set #1 analyses. The former is a small lake used for experimental introductions of rainbow trout and Patagonian silverside (*Basilychthys microlepidotus*, Atherinidae) and was heavily fished, experimentally (Quiros and Baigun 1986). Cronometro Lake was suspected to have pesticide generated environmental stress (Quiros and Baigun 1986) while Blanco Lake is shallow, turbid, fluctuates considerably in surface area and depth, and was practically without fish. Escondido is a dammed lake used for saw-mill activities and was practically without fish. El Diamante lake, a high-mountain lake in central Andes Mountains, was also included in the regional analysis because of its altitude and its salmonid fish population.

TEMP ranged from 3 to 5°C in the high-mountain lake and the glacial lakes in the southern Andes and from 12 to 14.5°C in the northern Patagonian Plateau lakes and reservoirs (Quiros and Drago 1985). Salmonid species have been introduced from the beginning of the century onward in this set of lakes. Salmonids are presently known to have established breeding populations in some Patagonian lakes. Most glacial lakes in Patagonia and Tierra del Fuego have been successfully colonized by salmonids (Baigun and Quiros 1985; Quiros 1987). CPUE ranged from 0.2 to 400.0 kg/night. Fifty-eight percent of the fish biomass captured in our sampling was introduced salmonids. East of the Andes, there are many small lakes in which salmonid populations are maintained almost entirely by periodic stocking. Nevertheless, the fish community in the Patagonian Plateau lakes and reservoirs was dominated by indigenous percichthyds and atherinids. The climatic, edaphic, and morphological attributes of lakes, especially TEMP, explained the relative abundance of salmonids in Patagonia (Quiros and Baigun 1986; Quiros 1987).

Pampa Plain

Twenty-six lakes were sampled for fish in the Pampa Plain. Melincue Lake was only included in our set #1 analysis. It is a very turbid lake, subject to earth embankment construction activities during sampling, has the lowest A/A ratio and the highest TP content of the ARLARE set.

Lakes are shallow (0.7 to 5.2 m), and TEMP ranges from 13.8 to 16.5°C. CPUE ranged from 17.4 to 505.1 kg/night. Most of the fish captured in the sampling were species native to the Pampa Plain. Forty-five percent of biomass was the Argentinian silverside and the common carp was captured only in five lakes. The fish community composition was related to the amount of open water and vegetated areas and wetlands, and to the organic matter (R. Quiros, unpubl. data).

Ordination of Lakes and Reservoirs

Climatic, morphometric, and edaphic characteristics were highly interrelated in the ARLARE data base (Table 3). Nineteen variables of different hierarchical levels were used in principal components ordination analyses (Table 4). Edaphic variables are commonly related to or determined by climate and morphometry, but must be tested in each case (Quiros 1987). I am also conscious of the log-linear structure that I have imposed to data in the ordination analyses (Gauch 1982; Zimmerman et al. 1983; Bolter and Meyer 1986).

The first component (PC1) explained 54.6% of total variance and loaded highly and positively on ZMEAN and SDL, and negatively on nutrient and organic matter variables, TEMP and CHL (Table 4). This first component represents a contrast between shallow lakes and reservoirs with high nutrient concentrations, high total and dissolved organic matter, high phytoplanktonic biomass, and high mean annual air temperatures, and deep lakes and reservoirs with higher water transparency, higher elevations and latitudes, belonging to the calcium plus magnesium and carbonate plus bicarbonate chemical water type, and with high TN:TP ratios. The second component explained 13.8% of the total variance and loaded heavily and negatively on DOb and LAT, and positively on CHL:TP ratio. The second component contrasts lakes and reservoirs at high elevations, high mean annual air temperature and belonging to the calcium plus magnesium water type, with large lakes at high latitudes and high oxygen levels at the water–sediment interface.

A continuum of lakes and reservoirs was defined on the ordination space of the two first components (Fig. 2). However, three spatial groups were distinguishable depending on region. A first group (A) includes the lakes and reservoirs of Patagonia and Tierra del Fuego, and a second group (B) includes most of the reservoirs of the central-western and northwestern arid region. The central-western reservoirs without an oxygen deficit in the hypolimnion are positioned near the Patagonian lakes and overlapped with the small, shallow Patagonian lakes situated immediately west of the Andes Mountains. A third group (C) includes the very shallow lakes located on the Pampa Plain. The shallowest northwestern reservoirs in group B overlapped the Pampa Plain lakes in group C.

The third and fourth components explained 9.0 and 6.3% of total variance, respectively and represent particular characteristics of the Argentinian lakes and reservoirs, such as regional distribution of large lakes and reservoirs with high dissolved solids and $C1^-$ or SO_4^{2-} water type, and sites with both high TN:TP and CHL:TP ratios.

The lakes and reservoirs with the highest CPUE are included in a zone of the principal components space that have low and intermediate values of PC1 and PC2, respectively (Fig. 3). In that zone are lakes and reservoirs with the lowest mean depths, the highest nutrient concentrations, the highest mean annual air temperatures, and intermediate values of CHL:TP ratios. All the sites with CPUE>150 kg/night and many of those with CPUE>60 kg/night are included there. However, those lakes and reservoirs with favorable climatic, morphometric, and edaphic conditions for high fish biomass, but with low bottom dissolved oxygen, and those with very low CHL:TP ratios are excluded (Fig. 3).

Factors Related to Fish Biomass

Correlation analyses results show that lakes and reservoirs with the highest nutrient levels, the lowest mean depths, and the highest mean annual air temperatures, tended to have, as expected, higher fish biomass (Table 3). For unscreened data (set #1, $n = 106$; Fig. 4) ZMEAN explained 31% of CPUE variance, and TON, TP, CHL, and TEMP explained 29, 19, 23, and 9% of it, respectively. For set #2 ($n = 99$), CPUE variance explained by the same variables was higher than for set #1, and TON was the most important single variable (Table 3). In this data set, a series of sites with high nonalgal suspended solids and others with an extensive aquatic macrophyte development were still included. Many of the northwest region

TABLE 3. Matrix correlation coefficients between environmental and fish variables for ARLARE data set #2 ($n=99$), Area (A, km^2), mean depth (ZMEAN, m), mean annual air temperature (TEMP, °C), total dissolved solids (TDS, $g \cdot m^{-3}$), total phosphorus (TP, $mg \cdot m^{-3}$), total organic nitrogen (TON, μM), total nitrogen to total phosphorus ratio (TN/TP), total chlorophyl (CHL, $mg \cdot m^{-3}$), carbonate plus bicarbonate to chloride plus sulfate (A/A), calcium plus magnesium to sodium plus potassium (C/C), bottom dissolved oxygen concentration at sampling (DOb, $mg \cdot L^{-1}$), number of fish species at sampling (NUM), catch per unit effort of Atherinidae (CPUEA, kg/night per standard gillnets), total catch per unit effort (CPUE kg/night per standard gillnets), and frequency of atherinids at sampling (%ATE).

Variable	A	ZMEAN	TEMP	TDS	TP	TON	TN/TP	CHL	A/A	C/C	DOb	NUM	CPUEA	CPUE	%ATE
A	1														
ZMEAN	0.41[a]	1													
TEMP	−0.16	−0.57[a]	1												
TDS	−0.01	−0.69[a]	0.64[a]	1											
TP	−0.18	−0.82[a]	0.64[a]	0.77[a]	1										
TON	−0.26[c]	−0.86[a]	0.59[a]	0.76[a]	0.89[a]	1									
TN/TP	0.02	0.49[a]	−0.50[a]	−0.51[a]	−0.81[a]	−0.46[a]	1								
CHL	−0.27[c]	−0.80[a]	0.72[a]	0.67[a]	0.82[a]	0.86[a]	−0.51[a]	1							
A/A	−0.23	0.34[b]	−0.46[a]	−0.78[a]	−0.36[b]	−0.36[b]	0.20	−0.33[b]	1						
C/C	−0.03	0.66[a]	−0.45[a]	−0.81[a]	−0.79[a]	−0.78[a]	0.54[a]	−0.59[a]	0.48[a]	1					
DOb	0.28[c]	0.19	−0.63[b]	−0.21	−0.23	−0.24	0.13	−0.41[a]	0.12	0.00	1				
NUM	0.19	−0.34[b]	0.43[a]	0.28[c]	0.37[b]	0.39[b]	−0.21	0.39[a]	−0.19	−0.29[c]	−0.13	1			
CPUEA	−0.17	−0.49[a]	0.52[a]	0.56[a]	0.52[a]	0.55[a]	−0.29[c]	0.66[a]	−0.35[b]	−0.49[a]	−0.14	0.18	1		
CPUE	−0.15	−0.68[a]	0.32[b]	0.51[a]	0.64[a]	0.69[a]	−0.36[a]	0.61[a]	−0.25	−0.60[a]	0.07	0.37[b]	0.62[a]	1	
%ATE	−0.22	−0.29[c]	0.50[a]	0.44[a]	0.33[b]	0.33[b]	−0.22	0.51[a]	−0.34[b]	−0.28[c]	−0.28[c]	−0.09	0.57[a]	0.28[c]	1

[a] t-test $p<0.0001$.
[b] t-test $p<0.001$.
[c] t-test $p<0.01$.

TABLE 4. Eigenvalues, percent variance explained and factor loadings for principal components analysis with 19 variables defined in the text, for set #2 ($n=99$).

Factor	I	II
Eigenvalue	10.38	2.63
Percent variance explained	54.6	13.8
A	0.19	−0.44
ZMEAN	0.87	0.01
SDL	0.89	−0.04
COL	−0.79	−0.15
TEMP	−0.79	0.42
ALT	0.59	0.50
TA	−0.92	−0.17
K20	−0.88	−0.12
HARD	−0.75	0.07
TP	−0.95	−0.13
TON	−0.92	−0.04
TOC	−0.93	−0.06
CHL	−0.88	0.28
LAT	0.41	−0.76
A/A	0.53	0.08
C/C	0.81	0.40
CHL:TP	−0.12	0.68
TN:TP	0.64	0.20
DOb	0.33	−0.76

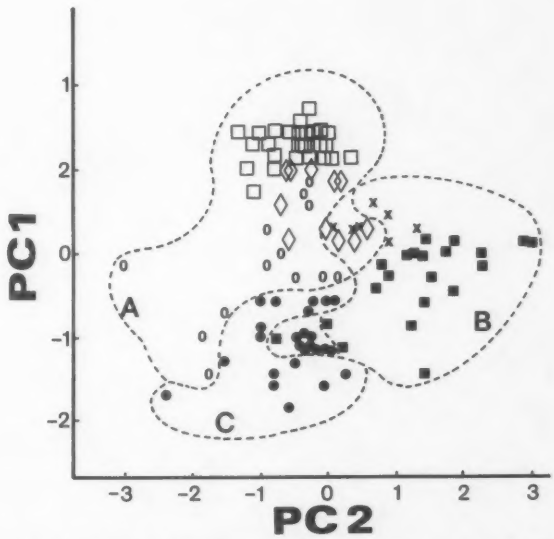

FIG. 2. Position of Argentinian lakes and reservoirs in the two first principal components space. (A). Patagonia and Tierra del Fuego. (□) Andean lakes; (◇) small lakes located east of the foothills of the southern Andes; (○) Patagonia Plateau lakes and reservoirs. (B) Arid region. (■) northwestern lakes and reservoirs; (X) central-western reservoirs. (C) Pampa Plain lakes (●).

reservoirs had an anoxic hypolimnion or low dissolved oxygen levels (<2.0 $mg \cdot L^{-1}$), a summer characteristic of most of the northwest arid region deep reservoirs. These reservoirs are monomictic in the spring only (Bonetto et al. 1976; Viana and Quiros 1989). When screening sites where CHL/TP<0.05 or DOb<2.0 $mg \cdot L^{-1}$ (set #3, $n=74$), the CPUE variation explained by both TON and TP increased (Table 5). Half of the reservoirs were screened in this step, but TEMP was still negatively related to DOb ($r=-0.54$, $P<0.001$).

System surface area ranged over six orders of magnitude in the ARLARE data base. Although A and ZMEAN were correlated (Table 3), there was no highly significant relationship between CPUE and A in any analyzed case. Similar results were obtained for the relationship between fish biomass and mean hydraulic residence time ($r=-0.05$, $n=43$). The same as TEMP, both surface (*Ts*) and bottom (*Tb*) water temperature at sampling were significantly related to CPUE.

Stepwise regression analysis was used to identify the environmental and fish variables that take account of CPUE variability and to quantify their relative magnitude. Most of the

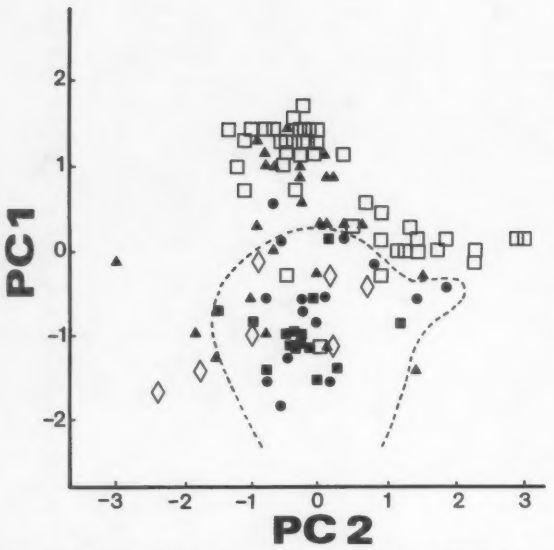

FIG. 3. Position of lakes and reservoirs in the two first principal components space and catch per unit effort (CPUE, kg/night per standard gillnets). (■) CPUE >150; (●) 150> CPUE >60; (▲) 60> CPUE >20; (□) 20> CPUE; (◇) lakes and reservoirs removed from main analyses.

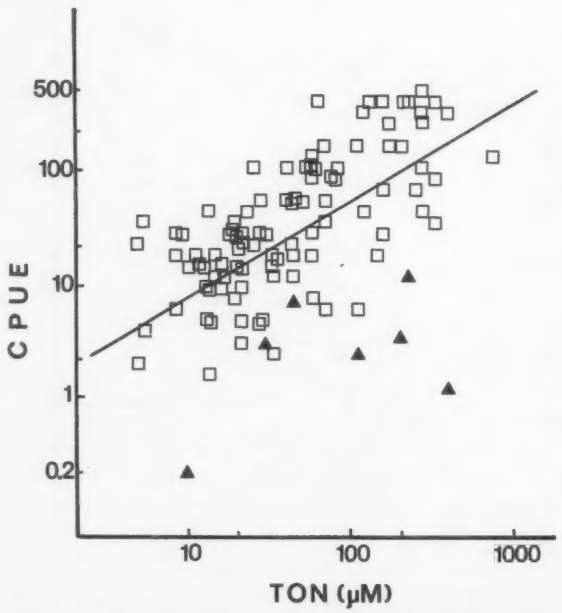

FIG. 4. Relation between fish biomass (CPUE, kg/night per standard gillnets) and mid-summer total organic nitrogen (TON, μM) for 106 lakes and reservoirs. (▲) lakes and reservoirs removed from main analyses.

lakes and reservoirs with DOb<2.0 mg·L^{-1} or CHL/TP<0.05 deviate negatively from the CPUE–TP (Fig 5) and CPUE–TON regressions. As was stated before, TON was the most important variable to explain CPUE variability in data set #2 (n=99). When DOb was introduced in the regression equation, CPUE variance explained improvement by 5% (Table 5 and 6). The mean value of the residuals to the regression of CPUE against TON were positive for the geographic data sets Patagonia (#PAT+1) and Pampa Plain (#PAM), and negative for the central and northwestern (#NOR-1) set where most of the sites with low oxygen levels in their hypolimnia were. However, the introduction of DOb in the regression equation diminished the residual mean values but did not modify that situation. CHL and ZMEAN were highly correlated to TON (Table 3), therefore, to avoid collinearity problems, they were forced not to enter to the regression equation.

Fish community composition and number of fish species at sampling take account of some CPUE residual variability after nutrients and dissolved oxygen. Both %ATE or NUM were positively related to the residuals of CPUE on TON and DOb and accounted for an additional 4% in CPUE explained variance (Table 6). Their covariation with both nutrient and oxygen variables was low (Table 3), and the inclusion in CPUE regression models has the advantage of improving fit.

Low chlorophyll to total phosphorus ratio resulted in lower fish biomass than predicted for most heterogeneous data sets. When CHL/TP was forced into the equation after TP, it improved the CPUE explained variability by 7%. But unlike CHL, it was not highly related to TP or DOb (r^2 = 0.01 and r^2 = 0.14, respectively). Besides, DOb and CHL/TP effects on CPUE-TP or CPUE-TON equations appear not to have a continuous character. Most of residuals were negative when DOb<2.0 mg·L^{-1} or CHL/TP<0.05, but were random distributed for DOb>2.0−1 mg·L^{-1}or CHL/TP>0.05. These facts justified the data screening that have conduced from set #2 (n=99) to set #3 (n=74). CPUE variability was more explained in single variable models for the latter, more homogeneous data set (Table 5) than in multiple regression models for the former (Table 6). TN/TP ratio was the most important variable to explain the residual variance in CPUE after TON, when most of the variability due to inorganic turbidity, macrophyte abundance, and hypolimnetic oxygen deficits had been removed (#3, n=74), and ZMEAN had been forced not to enter to the equation. But it only marginally improved CPUE explained variance in 2% (Table 6). Similar results were obtained when TN/TP was introduced after TP, but they were highly related (Table 3).

When lakes and reservoirs were considered separately, the level of CPUE variance explained was improved for lakes (Table 5, #2LA, n=70). For reservoirs the CPUE variability explained was lower when all the variables were considered (Table 5, #2RE, n=29). When screening was applied to lake data subset, the level of CPUE variance explained increased, and TP was the most important single variable to explain CPUE variation after screening for CHL/TP<0.05, DOb<2.0 mg·m^{-3} and TN/TP<37 (#3LA, n=47, Table 5). Reservoir differences with lakes appear to be more related to the facts that half of them have hypolimnia devoid of oxygen, introduced fishes, or suffer human influences that I cannot quantify here, than to its own characteristics as reservoirs.

Stepwise regression models for lakes were qualitatively similar to those for total sets, but CPUE variance explained was comparatively higher (Table 6). For most homogeneous lake set (#3LA, n=47), ZMEAN, %ATE or NUM only marginally improved CPUE explained variance (Table 5 and 6). However, NUM also appear to be a discontinuous character variable; sum of residuals to CPUE-TP regression for NUM=1 lakes was

TABLE 5. Simple regression models relating catch per unit effort (CPUE, kg/night per standard gillnets) to total organic nitrogen (TON, μM), total phosphorus (TP, mg · m^{-3}, total chlorophyll (CHL, mag · m^{-3}), mean depth (ZMEAN, m) and mean annual air temperature (TEMP, °C), for different levels of data screening. All the variables except TEMP were log transformed. Intercept (a), slope (b), and the coefficient of determination (r^2) are presented. LA = lakes, RE = reservoirs. F-test, $P < 0.001$.

	Independent variable				
	TP	TON	CHL	ZMEAN	TEMP
			set #2 (n = 99)		
a	1.695	0.226	2.769	4.886	2.245
b	0.513	0.831	0.427	−0.625	0.084
r^2	0.404	0.475	0.372	0.464	0.100[a]
			set #2LA (n = 70)		
a	1.839	0.250	2.942	4.987	1.838
b	0.530	0.856	0.501	−0.616	0.182
r^2	0.502	0.581	0.586	0.543	0.375
			set #2RE (n = 29)		
a	1.588	1.099	2.588	4.316	1.513
b	0.397	0.503	0.165	−0.524	0.086
r^2	0.168[b]	0.110[c]	0.039[c]	0.147[b]	0.066[c]
			set #3 (n = 74)		
a	1.540	0.124	2.863	5.182	2.112
b	0.657	0.917	0.514	−0.675	0.135
r^2	0.598	0.607	0.539	0.573	0.224
			set 3LA (n=47)		
a	1.499	−0.093	3.010	5.251	1.695
b	0.761	0.983	0.558	−0.659	0.216
r^2	0.727	0.688	0.700	0.636	0.481

[a] F-test, $P < 0.01$.
[b] F-test, $P < 0.05$.
[c] F-test, non-significant.

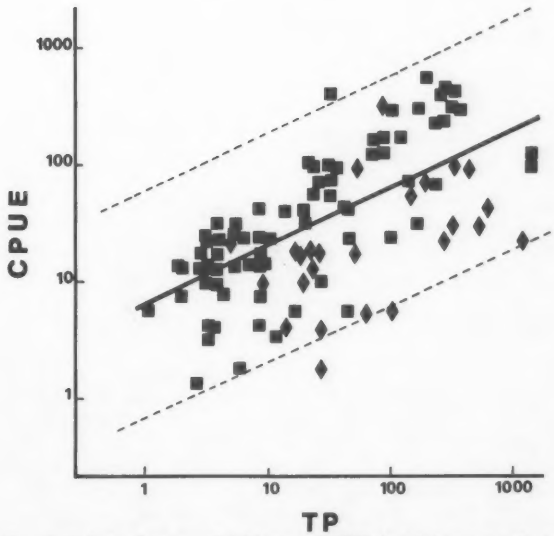

FIG. 5. Relation between fish biomass (CPUE, kg/night per standard gillnets) and mid-summer total phosphorus (TP, mg·m^{-3}) for data set #2 (n=99). (♦) lakes and reservoirs with DOb < 2 mg·L^{-1} (bottom dissolved oxygen) or CHL:TP < 0.05 (chlorohyll to total phosphorus ratio). Broken lines show 95% confidence limits.

highly negative when compared with NUM>1 lakes. When lakes with NUM = 1 were removed, TP explained 76% of CPUE variability (#4LA, n = 37), and the inclusion of ZMEAN and DOb accounted for an additional 5%. In this last data set TEMP was still negatively related to DOb ($r = -0.45$, $P<0.001$), and mean frequency of salmonids at sampling was 0.44.

Simple regression models for CPUE were developed mainly to compare with existing models in the literature (Table 5). For CPUE against ZMEAN, there was no evidence against a coincident (n=99) regression model ($F(2,95) = 3.10 > F = 1.74$; $P = 0.05$), and the difference in means of residuals of regression models for lakes and for reservoirs was not significant ($F = 3.51 < F(1,96) = 3.94$; $P = 0.05$). For CPUE against TP a unique regression model is not adequate ($F(2,95) = 3.10 < F = 4.58$; $P = 0.05$), but there was no significant difference between slopes for lake and for reservoir models ($F = 0.37 < F(1,95) = 3.90$; $P = 0.05$). TA was the most important single variable to explain CPUE variability in reservoirs, before DOb<2.0 mg·L^{-1} screening (loge CPUE = 2.509 + 0.955 (SE = 0.279) loge TA; $r = 0.30$, $n = 29$, RMSE = 1.025, $P = 0.002$). For total sets and for lakes, CPUE variance explained and regression equation slope increased with the screening process (Table 5). The slopes were in general higher for the lake subset than for the reservoir subset equations.

There were no significant differences between regression models of fish biomass on environmental variables for sites with and without atherinids. Atherinids at sampling (% ATE) was highly correlated with TEMP, CHL, and TDS (Table 3), and correlation coefficients practically did not change with data screening. For set #3 (n = 74) two subsets, with (#ATE) and without (#NOATE) atherinids, were considered. CPUE was most related to TEMP, TP, and CHL in the #ATE subset, and to ZMEAN and TON in #NOATE, but in each case there was no evidence against a coincident simple regression model. F-ratio was 0.03, 0.47, 1.39 and 1.50 for TON, TP, ZMEAN, and CHL models, respectively against $F(2,68) = 3.12$

TABLE 6. Multiple regression equations relating CPUE (kg/night per standard gillnets) to total organic nitrogen (TON, μM), total phosphorus (TP, mg · m^{-3}), mean depth (ZMEAN, m), bottom dissolved oxygen (DOb, mg · L^{-1}), chlorophyll to total phosphorus ratio (CHL:TP), total nitrogen to total phosphorus ratio (TN:TP, molar basis), and frequency of atherinids (%ATE). The coefficient of determination (R^2), and the root mean square error of regression (RMSE) are presented.

Equation	R^2	RMSE	F^a
set #2 (n = 99)			
loge CPUE = −0.764 + 0.903 loge TON + 0.102 DOb	0.534	0.966	54.97
loge CPUE = 1.079 + 0.595 loge TP + 0.142 DOb	0.524	0.982	34.87
loge CPUE = −0.968 + 0.769 loge TON + 0.119 DOb + 0.651 %ATE + 0.120 NUM	0.568	0.941	30.86
loge CPUE = 3.191 − 0.550 loge ZMEAN + 0.109 DOb + 0.806 %ATE + 0.152 NUM	0.562	0.947	30.11
set #2LA (n = 70)			
loge CPUE = −1.013 + 0.943 loge TON + 0.112 DOb	0.619	0.897	54.31
loge CPUE = −0.617 + 0.918 loge TON + 0.128 DOb + 0.213 loge CHL/TP	0.646	0.870	40.20
loge CPUE = −0.865 + 0.766 loge TON + 0.110 DOb + 0.780 %ATE + 0.117 NUM	0.656	0.865	30.91
set #3 (n = 74)			
loge CPUE = 1.921 + 0.819 loge TON − 0.331 loge TON/TP	0.627	0.904	59.79
set #3LA (n = 47)			
loge CPUE = 1.340 + 0.639 loge TP + 0.644 %ATE + 0.112 NUM	0.756	0.740	44.37

[a] F-test, $P \ll 0.01$.

TABLE 7. Correlation coefficients (r) of chlorophyll to total phosphorus (CH/TP), total zooplankton biomass to chlorophyll (TOZOO/CHL), fish biomass to chlorophyll (CPUE/CHL), and fish biomass to total zooplankton biomass (CPUE/TOZOO) ratios, with several variables defined in the text, for ARLARE data set #2 (n = 99).

	TOZOO/CHL	CPUE/TOZOO	CPUE/CHL	CHL/TP
ZMEAN	0.07	0.38[b]	0.40[a]	−0.15
TEMP	−0.39[b]	−0.33[b]	−0.63[a]	0.29[c]
TP	−0.17	−0.37[b]	−0.47[a]	−0.08
TON	−0.16	−0.37[b]	−0.47[a]	0.15
CHL	−0.46[a]	−0.36[b]	−0.72[a]	0.50[a]
TN:TP	0.13	0.24	0.33[b]	0.33[b]
DOb	0.33[b]	0.33[b]	0.58[a]	−0.38[b]
%ATE	−0.34[b]	−0.12	−0.39[b]	0.38[b]

[a] t-test, $P < 0.001$.
[b] t-tst, $P < 0.001$.
[c] t-test, $P < 0.01$.

($P = 0.05$). Similar results were obtained for set #2 ($n = 99$) analysis.

Factors Related to CPUE:CHL and CPUE:TOZOO Ratios

Both fish/phytoplankton and fish/zooplankton biomass ratios were negatively related to nutrient concentrations and temperature, and positively related to mean depth (Table 7). CPUE and CHL spanned three and four orders of magnitude, respectively, for the ARLARE data set #2 (Table 1), and CPUE:CHL ratio ranged from 0.11 to 116. It was higher for Patagonia (from 1.5 to 115.7), than for Pampa Plain and arid regions (from 0.31 to 22.9, and from 0.11 to 33.4, respectively). CHL has been the most relevant variable related to CPUE:CHL variation in the studied lakes and reservoirs (Table 5, Fig. 6a). The negative relationship was maintained for most homogeneous data sets (Table 5). The analysis of the limits of CPUE:CHL distribution for untransformed data clearly shows negative dependence on CHL. The 25 lakes and reservoirs that have CPUE:CHL>30, have CHL<2.5 mg·m^{-3}, and the 10 lakes with CPUE:CHL<6 also have CHL>60 mg·m^{-3}.

For relative fish biomass to total zooplankton biomass ratio (CPUE:TOZOO) the pattern of variable dependence was qualitatively similar to that obtained for CPUE:CHL ratio but relationships were weaker (Table 7). TOZOO explained 48% of CPUE variation for data set #2 (loge CPUE = 1.381 + 0.494 (SE = 0.059) loge TOZOO; $n = 99$, $F = 69.89$, $P << 0.001$) and 46% for #3LA (loge CPUE = 1.685 + 0.458 (SE = 0.074) loge TOZOO; $n = 47$, $F = 38.62$, $P < 0.001$). Obviously, CPUE:TOZOO diminished when TOZOO increased (Fig. 6b).

Discussion

Catch per unit effort, as estimated by gillnetting, is not an unbiased estimate of fish biomass. It is possible that relative fish biomass bias increases with the true biomass; this will result in the slope of the log–log equations for CPUE being below the true slope by an amount for any correlate. However, for the most homogeneous sets, simple regression equations for relative fish biomass have slopes similar to equations for yield and fish biomass developed from northern hemisphere lake comparisons. In the CPUE against TON regressions, the slopes were in general not significantly different from one. Similar results were shown by Hrbacek(1969) for fish yield and Kjeldahl nitrogen relationships in ponds. The TP exponent in the CPUE on TP models for most homogeneous sets (Table 5) ranged from 0.66 to 0.72 for total data and from 0.70 to 0.76 for lakes; it is 0.71 for the Hanson and Legett (1982) equation of fish standing crop on total phosphorus, and 0.70 for Lee and Jones (1981) equation of fish yield on phosphorus load, developed with Oglesby's (1977) fish data. However, CHL exponent in CPUE

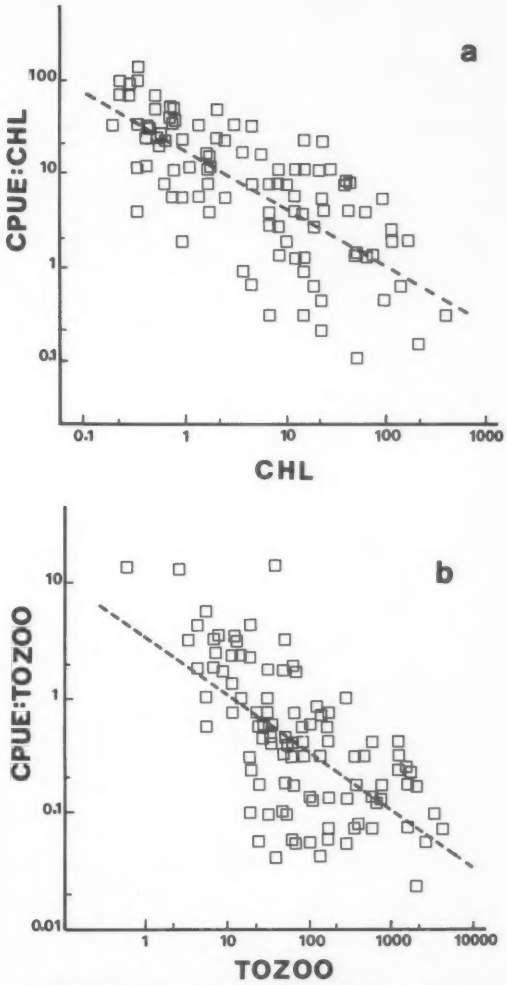

FIG. 6. Relation between (a) CPUE:CHL ratio (kg·m^{-3}/ g·night per standard gillnets) and mid-summer total chlorophyll (CHL, mg·m^{-3}, (b) CPUE:TOZOO (kg.pdL per μg dry weight per night per standard gillnets) and total zooplankton biomass at sampling (TOZOO, μg dry weight·L^{-1}), for Argentinian lakes and reservoirs (set #2, $n = 99$).

on CHL equations was 0.56 for my most homogeneous lake sets and was 1.17 for Oglesby's (1977) fish yield on chlorophyll regression equation. For the Oglesby (1977) data as well as in the Melack (1976) tropical lakes, fish yield/primary production ratio is positively related to ecosystem productivity. But for fish aquaculture ponds fish yield/primary production and fish yield/phytoplankton ratios were shown to be inversely related to primary production and phytoplankton, respectively (Hrbacek 1969; Almazan and Boyd 1978), and in an American midwestern set of lakes and reservoirs (Jones and Hoyer 1982), sport fish harvest was linearly related to chlorophyll. As was shown here for relative fish biomass, before and after nutrient condition had been taken into account, a direct relationship between fish standing crop and number of fish species was shown by Carlander (1955) for American midwestern reservoirs.

The absence of a relationship between fish biomass residual variability and mean annual air temperature, after nutrients and morphometry have been accounted for, could be related to the presence of aggregations of loosely associated fish species in the west-central and northwest, and in most of the southern Patagonia lakes and reservoirs. Salmonids in the Southern Hemisphere have similar temperature distribution patterns to the Northern Hemisphere. Summer temperature in both the epi- and hypolimnia and summer hypolimnion dissolved oxygen have been shown as factors that limited salmonid distribution in Argentinian lakes and reservoirs (Quiros 1987), and an inverse relationship between temperature and dissolved oxygen at the water–sediment interface was shown here, even for the most homogeneous lake sets. Salmonid introductions in early depauperate systems and the stocking of atherinids from the shallow, highly eutrophic, usually unstratified, and well oxygenated bottom waters of the Pampa Plain lakes, into the usually deep eutrophic, stratified in summer, with oxygen absent hypolimnion northwestern reservoirs, contribute to the condition of fish community homogeneity, for energy dependence for fish production, and in lakes under other constant environmental conditions as established by Ryder (1982), is violated for the ARLARE set. It could be that increasing energy inputs to aquatic systems, as measured in terms of mean annual air temperature, also will increase fish production rates, either through more rapid growth or decreased generation times (Ryder 1982), but have less influence, ultimately, on the size of standing crop of lake organisms (Northcote and Larkin 1956). Also I cannot reject a fish sampling bias, or a fishing gear selectivity effect. How much they also depend on nonlinearities between dependent and independent variables in data, is related to environmentally unsuitable conditions for fish (Christie and Regier 1988), or are related to system characteristics not yet analyzed, is something that needs to be investigated further.

External characteristics to the aquatic system, or directly determined by them, as total nutrient content, has been shown here as the most relevant to account for total fish biomass. Total organic matter, total phosphorus, and mean depth have been revealed as the most important for explaining variability in relative fish biomass among Argentinian lakes and reservoirs. On the other hand, internal variables, related to the dynamics of individual systems, lightly improved fish biomass explained variation in multiple regression models. The relationships between fish biomass and external variables, unlike that with internal variables, were maintained in spite of the change in set homogeneity. The results support the hypothesis that fish/phytoplankton, and fish/zooplankton biomass ratios are negatively related to lake trophy (Sprules et al. 1983), but they could be influenced by some effects and bias due to the fitting of the introduced fish species to their local environment and the related fish biomass, and is worthy of further attention. As distinguished from other extensive limnological and fish surveys (Northcote and Larkin 1956; Jenkins and Morais 1968), a relatively high proportion of fish biomass variability was explained. That could be related to a high level of interrelations among climate, morphometric, and edaphic factors, and also to the very wide range of variation of variables, for the ARLARE set.

Management Implications

The delimitation of a zone of the environmental principal components space with the highest relative fish biomass might

provide a useful direction for fisheries management, as a first approximation answer to fish biomass or yield problems, in areas where many lakes occur or in countries where funding for individual management of lakes and reservoirs is limited (Regier and Henderson 1980; Henderson et al. 1987). The ARLARE results show that very shallow Pampa Plain lakes are the most productive and also have the highest fish biomass. On the contrary, very deep, southern lakes of glacial origin have the lowest fish biomass. Lakes and reservoirs in the Patagonian Plateau and small and shallow lakes east of the southern Andes have intermediate fish biomass as well as central-western reservoirs. Very shallow northwestern reservoirs have limnological conditions and fish biomass comparable to Pampa Plain lakes.

Management objectives in Argentina have been usually directed to manage for sport fisheries. Anglers preferences have governed salmonid introductions and silverside spread in most lakes and reservoirs (Baigun and Quiros 1985). The traditional practices of stocking have continued for more than 80 yr with little question and no serious investigations about their results (Quiros 1987). Management strategies should reflect both the uses for which fish production is intended by man and the ecological realities of aquatic systems. Therefore, they would not be the same for different geographic regions.

Southern lakes of glacial origin were depauperated of fish species before the introduction of salmonids (Ringuelet 1975), and possibly fish production was lower. Native species of the families Aplochitonidae and Galaxiidae were practically absent in our sampling (Quiros and Baigun 1986). There are no studies about interrelationships between those fishes and salmonids in lakes. Because the predicted biomass for most of the lakes with very few number of species is higher than biomass at sampling, those lakes would be capable of supporting more fish. The shallow lakes in the Pampa Plain are practically the only Argentinian lakes with a relatively high fish community diversity (Ringuelet 1975). Some of them are subject to stress caused by human activities, and also have a changed fish community composition. For those lakes the predicted biomass is higher than biomass at sampling; before rehabilitation is attempted or silverside stocking is continued, it is recommended that a broad environmental assessment be made (sensu Ryder and Kerr 1988). The hypolimnia of most of the deep northwestern reservoirs with low levels of dissolved oxygen in summer, have no suitable conditions for their introduced fishes. Many of them had biomass at sampling lower than predicted biomass. Before any attempt of fish rehabilitation by stocking is made, an assessment of eutrophication causes is recommended.

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Evaluation of Models Predicting Mixing Depth

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Since mixing depth affects many aspects of lake productivity, including nutrient recycling, I evaluated the predictive power of 17 empirical models that relate mixing depth to morphometric variables to identify the best predictor. These models were tested empirically by compiling data from 123 temperate lakes of differing morphometry, geometry, and trophy. Four statistical indices of precision and bias, indicate that the model published by Shuter et al. (1983) using maximum effective length of the lake was the best published model for predicting mixing depth, although it is slightly biased. I then examined the effect of alternate predictors, reflecting lake configuration, basin shape, and geographical indices, to formulate an improved model. The best single predictor of thermocline depth (*THER*) was maximum effective length (*MEL*): Log *THER* = 0.336 Log *MEL* − 0.245. No improvement in predictive power was obtained by combining other variables. This model is statistically superior to that of Shuter et al. (1983) beause it is not biased, it represents a greater number of lakes, and it covers a broader range of lake sizes and shapes over a more extensive geographical region. Two other models, using lake area and length of shoreline are proposed as alternate predictive tools, if *MEL* is not readily available.

Étant donné que la profondeur de la zone de mélange affecte de nombreux aspects de la productivité lacustre, y compris le recyclage des éléments nutritifs, l'auteur a évalué le pouvoir de prédiction de 17 modèles empiriques qui lient la profondeur de la zone de mélange à des variables morphométriques, ce dans le but d'identifier la meilleure variable de prédiction. Ces modèles ont été testés empiriquement sur une compilation de données comprennent 123 lacs tempérés de morphométries, géométries et niveaux trophiques divers. Quatre indices statistiques de précision et de biais identifient le modèle publié par Shuter et al. (1983), utilisant la longueur effective maximale du lac, comme étant le meilleur modèle de prédiction de la profondeur de la zone de mélange, même s'il est légèrement biaisé. L'auteur a par la suite examiné l'effet d'autres variables explicatives reflétant la configuration du lac, la forme du bassin ainsi que des indices géographiques afin de formuler un modèle amélioré. La meilleure variable explicative de la profondeur de la thermocline (*THER*) est la longueur effective maximale (*MEL*) : Log *THER* = 0.336 Log *MEL* − 0.245. Le pouvoir de prédiction n'est nullement amélioré par l'ajout d'aucune autre variable. Ce modèle est statistiquement supérieur à celui de Shuter *et al.* (1983) parce qu'il n'est pas biaisé, qu'il a été formulé à partir d'un plus grand nombre de lacs et qu'il couvre une gamme plus importante de tailles et de formes de lac, et ce, sur une étendue géographique plus vaste. Deux autres modèles utilisant la superficie du lac et la longueur du rivage sont proposés comme outils alternatives de prédiction lorsque n'est pas aisément disponible.

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One of the most important physical characteristics of a lake is whether it stratifies seasonally, and if so, the depth to which mixing is limited by stratification. Mixing depth controls nutrient recycling and the biological productivity of the aquatic ecosystem (Ohle 1952; Fee 1979; Patalas 1984). Stratification influences the nature of the habitat for organisms and therefore their depth distribution (Gorham and Boyce 1980). The thermocline isolates the deeper hypolimnion from the heat sources at the surface, thus greatly reducing the heat transfer into that portion of the lake. It also isolates the profundal benthos from the nanoplankton (Jonasson 1972) and greatly influences the oxygen regime.

Mixing depth has attracted attention since the early years of limnology. Ruttner (1931); Yoshimura (1936); and Berger (1955) pointed out that mixing depth increased as a power function of lake size. Subsequently, a number of models have related mixing depth to different morphometric parameters.

Such models are needed to quantify the relationship between the depth of the mixed layer and the vertical distribution of phytoplankton (Talling 1971). Predictive models of phytoplankton depth distribution require knowledge of mixing depth since the presence and depth of the metalimnetic chlorophyll peak depends on sufficient radiation penetrating to a low turbulence zone (Fee 1976). A simple prediction of mixing depth is also required by models such as Ragotzkie's (1978), in order to estimate the heat content of stratified lakes. Unless this information can be obtained more quickly than by repeated vertical temperature soundings, the entire purpose of a heat budget synthesis from minimal data would be defeated.

The primary objective in this study was to assess the accuracy of 17 empirical models that predict the depth of the mixed layer from morphometric variables, over a wide range of lakes. Subsequently, I examined the effect of alternate morphometric predictors and geographical indices to provide both insight into the effects of lake morphometry on the determination of the depth of mixing and to formulate an improved model.

Methods

This work was based on literature data from 113 North American lakes including data collected from 14 lakes of the

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TABLE 1. Sources of data used in the model evaluation analyses.

Region	Number of lakes	Data source
Canada	46	
British Columbia	20	Stockner and Shortreed 1974, 1983
Ontario	10	Cruikshank 1984; Fee 1976, 1979; Fee et al. 1977; McQueen and Lean 1983; D. J. McQueen (unpubl. data)
Quebec	16	M. Hanna (unpubl. data); Leclerc 1979; Zettler and Carter 1986
United States	67	
California	1	Abbot et al. 1984; Goldman and De Amezaga 1975; Wetzel 1975
Maine	17	Davis et al. 1978
Minnesota	47	Ford and Stefan 1980; Osgood 1982, 1984 a, b, 1985
Wisconsin	2	Juday and Birge 1941; Ness and Bunge 1956; Rawson 1955; Stauffer and Lee 1973; Wetzel 1975
Elsewhere	10	
Denmark	1	Hargrave 1975; Jonasson 1972
England	2	Ramsbottom 1976; Talling 1971; Wetzel 1975
Israel	1	Serruya 1975
Italy	1	Bonacina and De Bernardi 1978; Calderoni and Mosello 1978; Ruggiu and Saraceni 1978
Netherlands	3	Dorgelo et al. 1981; Steenbergen and Verdouw 1982; Swain et al. 1987
New Zealand	2	Viner and Kemp 1983; White 1982

Eastern Townships and the Laurentians, Quebec (M. Hanna, unpubl. data), and 10 lakes from the rest of the world (Table 1). For some of these lakes, observations from more than one sampling site were included in the analysis. Only data from the period of maximum heat content of the lake (July–August) were compiled since stratification most distinctly represents lake characteristics at this time. The data comprise lakes of diverse morphometries (Table 2), levels of trophy, as well as different geographical locations (latitude: 32–55°N, 38°S, altitude: 10–1897 m) and therefore climatic conditions.

Mixing depth was defined as epilimnion depth and planar thermocline depth, the two most widely used terms. Epilimnion depth is the depth at which the temperature gradient is $<1°C \cdot m^{-1}$ during the period of greater stability (Welch 1948). In the few cases where "double epilimnia" were obvious, the deeper one was the more pronounced and was used for calculations. Planar thermocline depth is the depth of the maximum rate of temperature change per unit depth (Hutchinson 1957). Mixing depth was estimated from vertical temperature profiles measured at the deepest point in each lake, by in situ sampling at 1-m intervals (smaller intervals were used at steep temperature gradients) using a portable field meter (Leeds and Northrup, Model 7932) for the lakes sampled, and by digitizing temperature profiles obtained from the literature.

Morphometric data were obtained by digitizing bathymetric maps according to the definitions of Hakanson (1981). The morphometric variables digitized include: maximum length (*ML*), maximum width (*MW*), maximum effective length (*MEL*), maximum effective width (*MEW*), effective length (*EL*), fetch (*F*), area (*A*), and shoreline length (*L*). For regularly shaped lakes, maximum effective length (*MEL*) corresponds to maximum length (*ML*), whereas for irregularly shaped lakes, *ML* may be curvilinear, and thus greater than *MEL*. The variables compiled include: maximum depth (Z_{max}), mean depth (Z_{avg}), volume (*V*), latitude (*LAT*) and altitude (*ALT*). Other indices, such as shoreline development $D_L = L/[2\sqrt{\pi \cdot A}]$, volume development ($D_V = V/[1/3\ A \cdot Z_{max}]$), relative depth ($Z_{rel} = [50\ Z_{max} \cdot \sqrt{\pi}]/\sqrt{A}$) and the ratio Z_{avg}/Z_{max}, were calculated.

TABLE 2. Summary of the descriptive statistics of the morphometric variables and thermocline depth of the lakes studied. The variables include: maximum length (*ML*), maximum width (*MW*), maximum effective length (*MEL*), maximum effective width (*MEW*), shoreline length (*L*), shoreline development (D_L), area (*A*), maximum depth (Z_{max}), mean depth (Z_{avg}), relative depth (Z_{rel}), volume (*V*), volume development (D_V), and thermocline depth (*THER*).

Parameter	Units	Range	Mean	Median	*n*
ML	m	172.00 – 16 9000	26 000	5300	167
MW	m	113.00 – 82 600	4150	1360	167
MEL	m	172.00 – 10 8000	12 000	4120	167
MEW	m	113.00 – 29 500	3130	1410	167
L	m	523.00 – 36 5000	71 500	14 200	167
D_L		0.80 – 6	3	2	167
A	km^2	0.02 – 1050	73	4	167
Z_{max}	m	4.60 – 501	35	20	133
Z_{avg}	m	2.10 – 313	31	10	155
Z_{rel}		0.18 – 9	2	1	133
V	$10^6 m^3$	0.10 – 15 6000	6770	6	155
D_V		0.49 – 3	1	1	121
THER	m	2.64 – 34	11	9	167

Results

Relationships among Morphometric Variables

Descriptive statistics of the morphometric parameters are given in Table 2. This data set covers a wide range of lake sizes. The lakes for which morphometric data were obtained ranged over four to five orders of magnitude in their linear dimension and areas and over two orders of magnitude in their depths.

Most of the morphometric parameters are very highly intercorrelated (Table 3). The highest correlations are among any combination of: maximum length, maximum width, maximum effective length, maximum effective width, area, and length of shoreline, shoreline development, maximum depth, mean depth, relative depth, and volume, where the absolute value of *r* varies from 0.65 to 0.98. The highest correlation relates *MEL* to Area and length of shoreline. A second group of variables that are not significantly correlated to each other, includes: effective length, fetch, the ratio Z_{avg}/Z_{max}, and volume development.

Relationships between Morphometric Variables and Mixing Depth

Epilimnion depth is strongly related to thermocline depth (Fig. 1). The slope of that relation is not significantly different from 1 but the intercept (2.4) is significantly greater than 0 (*F*-test $p<0.05$), suggesting that planar thermocline depth is on average 2.4 m below the epilimnion.

Both epilimnion and thermocline depths were most strongly correlated with *MEL*, area and length of shoreline ($r = 0.85$ to 0.92), and significantly correlated with *ML*, *MW*, *MEW*, Z_{max}, Z_{avg}, Z_{rel}, D_L, and *V* (Table 3). No significant correlations exist between either measure of mixing depth and *EL*, *F*, D_V, Z_{rel},

TABLE 3. Correlation matrix of the logarithms of the morphometric variables and thermocline depth of the lakes studied. The variables include: maximum length (*ML*), maximum width (*MW*), maximum effective length (*MEL*), maximum effective width (*MEW*), effective length (*EL*), fetch (*F*), shoreline length (*L*), shoreline development (D_L), area (*A*), maximum depth (Z_{max}), mean depth (Z_{avg}), relative depth (Z_{rel}), volume (*V*), volume development (D_V), altitude (ALT), latitude (LAT), thermocline depth (*THER*), and epilimnion depth (EPI).

	ML	*MW*	*MEL*	*MEW*	*EL*	*F*	*L*	*A*	D_L	Z_{avg}	Z_{max}	Z_{rel}	*V*	D_V	ALT	LAT
n	167	167	167	167	28	28	167	167	167	142	107	107	142	107	55	128
ML	1.00															
MW	0.88	1.00														
MEL	0.93	0.84	1.00													
MEW	0.85	0.93	0.92	1.00												
EL	0.84	0.71	0.20	0.33	1.00											
F	0.89	0.65	0.48	0.48	0.83	1.00										
L	0.93	0.84	0.98	0.91	0.26	0.51	1.00									
A	0.93	0.88	0.98	0.95	0.33	0.55	0.98	1.00								
D_L	0.76	0.56	0.77	0.63	0.07	0.33	0.85	0.74	1.00							
Z_{avg}	0.72	0.59	0.76	0.67	−0.02	0.23	0.76	0.76	0.64	1.00						
Z_{max}	0.63	0.56	0.78	0.70	−0.06	0.21	0.74	0.74	0.54	0.91	1.00					
Z_{rel}	−0.64	−0.66	−0.65	−0.69	−0.53	−0.55	−0.68	−0.70	−0.37	−0.11	−0.15	1.00				
V	0.90	0.83	0.97	0.91	0.26	0.51	0.96	0.98	0.73	0.88	0.84	−0.59	1.00			
D_V	−0.11	−0.01	−0.22	−0.12	0.19	0.03	−0.26	−0.16	−0.50	−0.15	−0.26	0.04	−0.09	1.00		
ALT	0.13	0.18	0.29	0.33	0.09	−0.01	0.21	0.31	−0.10	0.65	0.55	0.14	0.44	0.16	1.00	
LAT	0.22	0.02	0.22	0.04	−0.49	−0.50	0.25	0.18	0.43	0.38	−0.17	0.41	0.25	−0.08	−0.08	1.00
THER	0.87	0.78	0.92	0.85	0.15	0.44	0.91	0.91	0.68	0.72	0.72	−0.62	0.90	−0.19	0.23	0.11
EPI	0.81	0.72	0.88	0.82	0.17	0.36	0.86	0.86	0.64	0.75	0.73	−0.49	0.88	−0.11	0.39	0.20

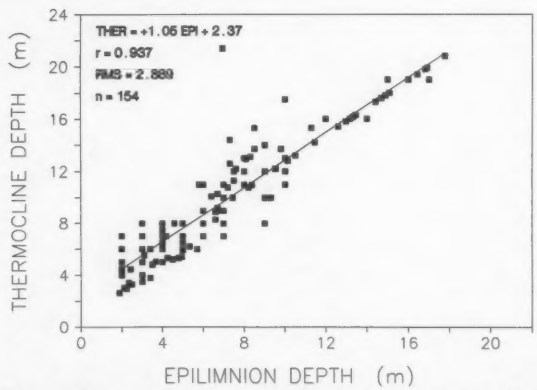

FIG. 1. Relationship between thermocline depth and epilimnion depth.

the ratio Z_{avg}/Z_{max}, *ALT* or *LAT*. In general, the correlations with morphometric parameters are slightly stronger for thermocline depth than for epilimnion depth. This is consistent with Cruikshank's (1984) findings. This difference likely results from the larger subjective element in the determination of epilimnion depth which is more sensitive to superficial heating, than thermocline depth. The definition of epilimnion depth cannot be applied to some lakes, e.g. lake Taupo, where the change of temperature is so gradual that a gradient of $1°C \cdot m^{-1}$ is never reached.

Model Evaluation

Seventeen empirical models for predicting mixing depth (Table 4), were statistically tested by comparing their abilities to predict mixing depths of the complete set of lakes. Most of these models are based on data from local groups of northern temperate lakes; they were therefore generated from lakes of similar climate, geography, size, and shape. Khomskis and Filatova (1972) provided an empirical formulae for the upper and lower metalimnion boundaries which uses a computed morphometric parameter "*N**", involving *ML*, *F*, Z_{max} and *MW*. The remaining models relate mixing depth (epilimnion or thermocline depth) to single morphometric parameters such as: *ML*, *MEL*, *A*, Z_{max}, *V*, and fetch defined as *MEL* (Cruikshank 1984; Shuter et al. 1983), (*MEL*+*MEW*)/2 (Patalas 1961, 1984), or $\sqrt{A}$ (Arai 1964, 1981).

Most models are cited as power functions of the form $Y = aX^b$, even though the coefficients were obtained from regressions on logarithmically transformed data such that: Log Y = Log a + b Log X. Fair appraisal of the predictive ability of the different models must use the log-transformed estimates.

The correlation coefficients relating the predictions of these models to the observed values (Table 5) distinguish two groups of models: those with strong correlations ($r = 0.87$ to 0.92) and those with lower correlations ($r = 0.41$ to 0.73). The stronger correlations involve models using *ML*, *MEL*, *MEW*, *A*, *V*, or some combination of these variables. Models with lower correlations use fetch or maximum depth as unique predictor variables, or a combination of *ML*, *F*, Z_{max}, and *MW* (Khomskis and Filatova 1972). The correlations are again slightly higher for thermocline depth than for epilimnion depth. The highest correlations between predicted and observed thermocline depths ($r = 0.92$) are given by the models using *MEL* (Shuter et al. 1983; Cruikshank 1984; Green et al. 1987). These correlations are not significantly better than those derived from models using (*MEL*+*MEW*)/2, volume or area (*F*-test, $p > 0.05$).

Model evaluation cannot be limited to the coefficient of determination, since it merely gives a measure of the linear association between the variables. The models were evaluated for precision and accuracy according to four criteria: (1) an *F*-test identifying the most precise models as those with the smallest *F*-values associated with the variance around the predicted values (*RMS*) (Table 6), (2) a *t*-test identifying unbiased models as those having residual means (ε) to the predicted values not significantly different from zero, (3) the lack of a trend in the distribution of the residuals associated with each model, and finally (4) the slope and intercept of the logarithmic regres-

TABLE 4. Summary of the most common published models predicting mixing depth. The models intended to predict thermocline depth are marked with an asterisk, the other models predict epilimnion depth. The listed values of r^2, number of lakes and units are those published with the original models.

Author/ Data Source	Region	Model	r^2	Number of lakes	Units
Straskraba 1980/ Yoshimura 1936	Japan	$3.56\ ML^{0.46}$	0.48	19	km
Green et al. 1987	New Zealand	$7.00\ MEL^{0.42}$	0.79	33	km
Shuter et al. 1983	North America	$*0.298 \ln F + 1.82$ $F = MEL$	0.66	73	km
Cruikshank 1984	ELA	$0.12\ F^{0.5}$ $F = MEL$	0.71	20	m
Straskraba 1980/ Patalas 1960, 61	Poland Baltic lowland	$4.55\ EL^{0.455}$ $EL = (MEL + MEW)/2$	0.88	53	km
Ventz 1973	East Germany Baltic lowland	$4.72\ EL^{0.39}$ $EL = (MEL + MEW)/2$		30	km
Patalas 1961	Northern Poland	$4.4\ F^{0.5}$ $F = (MEL + MEW)/2$	0.88	53	km
Patalas 1984	Poland, Central Canada	$4.6\ F^{0.41}$	0.85	88	km
	Yukon-Yellowknife	$F^{0.41}$ $F = (MEL + MEW)/2$		23	
Arai 1964	Japan	$6\ F^{0.33}$ $F = A^{0.5}$		32	km
Arai 1981	Japan	$6.22\ F^{0.304}$ $F = A^{0.5}$	0.53	36	km
Ragotzkie 1978	Wisconsin Central Canada	$4\ F^{0.5}$		18	km
Gorham and Boyce 1980	Worldwide	$*\ln((A)^{0.5}/43)^{2.35}$	0.66	150	m^2
Osgood 1987	TCMA, Minnesota	$3.02\ A^{0.5} + 1.108$	0.98	8	km^2
Cruikshank 1984	ELA	$1.54\ A^{0.28}$	0.64	20	km^2
Cruikshank 1984	ELA	$0.69\ Z_{max}^{0.56}$	0.48	20	m
Cruikshank 1984	ELA	$1.96\ V^{0.22}$	0.72	20	$10^5 m^2$
Khomskis and Filatova 1972	Worldwide	$Zu = 8.5\ N^*$ $Ze = 15\ N^*$ $N^* = \frac{ML \times F}{Z_{max} \times MW}$		70	m

sion relating the observed and predicted values had to be nonsignificantly different from zero and one, respectively (F-test) (Seber 1977) The values for these latter criteria are given in Table 5).

The models by Shuter et al. (1983) and Arai (1964, 1981) are the most precise, since the variances around the predicted regressions (*RMS*) are the smallest and their associated F-values are below the critical value (Table 6). Furthermore, t-tests on the residuals revealed that most models are biased, and of the three most precise models only that of Shuter et al. (1983) had a residual mean that is not significantly different from zero. However, since a residual mean of zero does not necessarily mean that the model is unbiased, further consideration of the distribution of the residuals is needed. A significant correlation ($r = 0.24$) between the values predicted by Shuter et al. (1983) and their residual errors indicates bias in the model, and this is confirmed by the regression between the predicted (Shuter et al. 1983) and observed thermocline depths (Fig. 2) which has an intercept and slope that are significantly different from 0 and 1, respectively (F-test, $p < 0.05$). Even though the model by Shuter et al. (1983) is biased, it is the best available, since it provides the best fit, and has the highest r, the lowest t-value, and despite the trend in the distribution of its residuals, its intercept and slope are the closest to 0 and 1, respectively.

All other models have much greater bias (t-values, Table 5). The models by Straskraba (1980); Cruikshank (1984); Ventz (1973); Patalas (1961, 1984); Arai (1964, 1981); and Gorham and Boyce (1980) tend to underestimate thermocline depths, while the model of Green et al. (1987) tends to overestimate them. The departures of the predictions based on the 16 other models might reflect the regional differences in the data used to generate the different models. The underestimations obtained by Arai's (1964, 1981) models might reflect the wind protection afforded by the small and steep watersheds of Japanese lakes. The discrepencies observed for models based on ELA (Cruikshank 1984) and TCMA (Osgood 1987) data, might be accounted for by the restricted ranges of lake sizes used to generate them. The deeper themocline depths of New Zealand lakes may reflect their maritime climate (Stout 1974; Jolly and Irwin 1975; Green et al. 1987).

A More Representative Model

The data set compiled in this study allows the development of a more general model, because it represents a larger and more diverse set of lakes. This analysis was carried out on a data set comprising 123 lakes, whereas the analysis by Shuter et al. (1983) only involved 73 lakes. The lake morphometry ranges covered in this study are also more extensive, they comprise larger (*MEL* up to 108 km versus 47.9 km) and deeper (Z_{avg} up to 313 m versus 69.5 m) lakes.

TABLE 5. Comparison of the logarithmic regressions between observed thermocline depths and the predictions given by the different models. Listed are the residual mean values (ε) observed minus predicted, the residual means that are significantly different from zero (*t*-test) with a probability <0.05 are marked with an asterisk, the intercept (*a*), the slope (*b*), the correlation coefficient (*r*), the *F*-test value (*F*-value) and associated probability (*P*) of the hypothesis that $a=0$ and $b=1$ (Seber 1977), and the sample number (*n*).

Author/ Data Source	Parameter	ε	*a*	*b*	*r*	*F*-value	*P*	*n*
Straskraba 1980/ Yoshimura 1936	*ML*	0.0587*	0.454	0.571	0.87	285.8	0.0000	167
Green et al. 1987	*MEL*	−0.1375*	0.087	0.790	0.92	58.8	0.0000	167
Shuter et al. 1983	$F=MEL$	−0.0031	−0.295	1.127	0.92	11.8	0.0000	167
Cruikshank 1984	$F=MEL$	0.0765*	0.373	0.672	0.92	223.3	0.0000	167
Straskraba 1980/ Patalas 1960,61	$EL = (MEL + MEW)/2$	0.0962*	0.285	0.786	0.92	65.9	0.0000	167
Ventz 1973	$EL = (MEL + MEW)/2$	0.1126*	0.184	0.917	0.92	7.3	0.0011	167
Patalas 1961	$F = (MEL + MEW)/2$	0.0885*	0.342	0.715	0.92	141.0	0.0000	167
Patalas 1984	$F = (MEL + MEW)/2$	0.1138*	0.224	0.872	0.92	19.1	0.0000	167
Arai 1964	$F=A^{0.5}$	0.0791*	−0.031	1.122	0.91	10.0	0.0001	167
Arai 1981	$F=A^{0.5}$	0.0732*	−0.125	1.218	0.91	26.9	0.0000	167
Ragotzkie 1978	*F*	0.0938*	0.838	0.174	0.41	141.9	0.0000	28
Gorham and Boyce 1980	*A*	0.3366*	0.525	1.291	0.89	33.4	0.0000	167
Osgood 1987	*A*	0.0169	0.553	0.443	0.91	7197.0	0.0000	167
Cruikshank 1984	*A*	0.5842*	0.718	0.661	0.91	220.7	0.0000	167
Cruikshank 1984	Z_{max}	0.3046*	0.475	0.715	0.71	21.1	0.0000	133
Cruikshank 1984	*V*	0.0557*	0.421	0.609	0.91	326.6	0.0000	155
Khomskis and Filatova 1972	$\frac{ML \times F}{Z_{max} \times MW}$	0.3217*	0.526	0.649	0.73	44.2	0.0000	133
Equation (1)	*MEL*	0.0000	0.000	1.000	0.92	0.0	1.0000	167

I also compiled variables which had not been examined previously, so that a number of alternative predictors could be considered. Since mixing depth is thought to be deeper in lakes that are narrower and shallower relative to their length (Khomskis and Filatova 1972; Straskraba 1980), I included shape factors that differentiate lake shapes, in order to test the hypothesis that mixing depth is also related to basin morphometry and not only to lake size. These shape factors are: D_L (degree of irregularity), Z_{rel} and Z_{avg}/Z_{max} (lake basin shape), D_V (lake basin form), MEL/MEW, $MEL/\sqrt{A}$, $MEW/\sqrt{A}$ (lake configuration), MEL/F and $\sqrt{A}/F$. Unlike most of the morphometric variables, these shape factors may be combined with *MEL* in a multiple regression model, without introducing the problem of collinearity which results from the combined use of variables that are highly correlated among themselves.

Different "all possible subset regressions" were performed on different combinations of this data set comprising all the logarithmically transformed morphometric parameters, including fetch, as defined by Hakanson (1981); Cruikshank (1984); Patalas (1961, 1984); and Arai (1964, 1981), the shape factors, and the geographical indices (*LAT* and *ALT*). The correlation coefficients showed strong dependence of mixing depth upon *ML*, *MW*, *MEW*, Z_{max}, Z_{avg}, Z_{rel}, and *V*.

The best single predictor of mixing depth remains *MEL* (Fig. 3a). Maximum effective length is defined by the straight line connecting the two most distant points on the shoreline over which wind and waves may act without interruptions from land or islands (Hakanson 1981). Logarithmic transformation was used to better satisfy the assumptions of linear regression (Box and Tidwell 1962): better linear fit and homoscedastic residuals. The addition of further variables in the regression equation removed less than 0.25% of the unexplained variation. The regression of observed versus predicted values yielded a slope and an intercept which are not significantly different from 1 and 0, respectively and there is no trend in the distribution of the residuals (Fig. 3b). This relation seems representative of temperate lakes and may therefore be used to estimate thermocline depths, since relationships which were not significantly different were generated by separate analyses of two randomly chosen subsets of the data.

Both Straskraba (1980) and Patalas (1984) state that relationships of this sort tend to overestimate thermocline depths of large lakes. They conclude that thermocline depth increases with lake size, up to $ML=25$ km, and thereafter remains constant at about 20–25 m. The models by Ventz (1973); Arai (1964, 1981); Gorham and Boyce (1980); Cruikshank (1984) using area and maximum depth, and Khomskis and Filatova (1972) overestimate thermocline depths of large lakes (paired *t*-test for lakes with $MEL>25$ km, $n=40$). Other models, such as those by: Straskraba/Yoshimura (1980), Cruikshank (1984) using *MEL* or volume, and Osgood (1987) tend to underestimate thermocline depths of large lakes. The model by Green et al. (1987) tends to underestimate all thermocline depths, but to a greater extent for smaller lakes. Equation (1) however offers unbiased predictions of thermocline depths of 20–35 m for lakes with *MEL* of up to 40 km. Beyond 40 km, further increases in *MEL* may have no effect in deepening of thermocline. A plot of the relationship between thermocline depth and MEL shows that thermocline depth increases with lake size, up to $MEL=40$ km and then seems to remain constant. Furthermore

TABLE 6. Comparison of the precision of the predicted values of mixing depth obtained by the different models. Listed are the variance around the predicted values (*RMS*), and the associated *F*-test values (*F*-value), computed as the ratio of *RMS* of the model to *RMS* of the 'best' model (Arai 1964, 1981) to indicate if the residuals are significantly greater. These values were computed from the data set used in this study.

Author/ Data Souce	Parameter	*RMS*	*F*-value
Straskraba 1980/ Yoshimura 1936	ML	0.0419	2.57
Green et al, 1987	MEL	0.0307	1.88
Shuter et al. 1983	$F=MEL$	0.0180	1.10
Cruikshank 1984	$F=MEL$	0.0262	1.61
Straskraba 1980/ Patalas 1960,61	$EL=(MEL+MEW)/2$	0.0219	1.34
Ventz 1973	$EL=(MEL+MEW)/2$	0.0221	1.36
Patalas 1961	$F=(MEL+MEW)/2$	0.0245	1.50
Patalas 1984	$F=(MEL+MEW)/2$	0.0230	1.41
Arai 1964	$F=A^{0.5}$	0.0163	1.00
Arai 1981	$F=A^{0.5}$	0.0163	1.00
Ragotzkie 1978	F	0.1186	7.28
Gorham and Boyce 1980	A	0.3369	20.67
Osgood 1987	A	0.0848	5.20
Cruikshank 1984	A	0.3654	22.42
Cruikshank 1984	Z_{max}	0.1158	7.10
Cruikshank 1984	V	0.0322	1.98
Khomskis and Filatova 1972	$\frac{ML \times F}{Z_{max} \times MW}$	0.1283	7.87
This paper	MEL	0.0086	0.53

the negative residuals obtained from the predictions of thermocline depth for lakes with $MEL>40$ km confirm that thermocline depths of these lakes are in fact shallower than would be expected from equation (1). To verify if the 11 sites from lakes with 40 km$<MEL<$108 km included in the analysis distort the final predictive equation, the *MEL* regression was repeated after the exclusion of these large lakes. The new relationship was not significantly different from equation (1), suggesting that the inclusion of larger lakes did not distort the predictive equation. Nevertheless, the relation cannot be linearly extrapolated beyond the limit of this data set.

The predictive power of *MEL* is marginally superior to that of area and shoreline length, but not significantly so. Thus, two other models using the logarithms of these variables have been generated (Table 7). These may be used as alternate predictors, if *MEL* is not readily available, however both are more difficult to obtain than *MEL*.

Shape factors significantly improved neither the correlation coefficient, nor the residual mean square, over the model using the single predictor log *MEL* (equation (1)). Latitude or altitude did not explain any of the residual variance. As Arai (1981) and Patalas (1984) concluded, the most important factor affecting the thermal structure of lakes within the temperate zone is lake size and the effect of climate is negligible. This can be concluded on the basis that similar mixing depths were observed for lakes in such remote latitudes (32–55°N) and altitudes (10–1897 m).

Comparison between this Model and that of Shuter et al. (1983)

A paired *t*-test showed that the predictions from equation (1) and those from the model of Shuter et al. (1983) differ significantly ($t=-32.32$) and an *F*-test showed that their regression coefficients are significantly different. Equation (1) is superior in that it is not biased and the distribution of the residuals more closely approximates a normal distribution (Wilk-Shapiro Test),

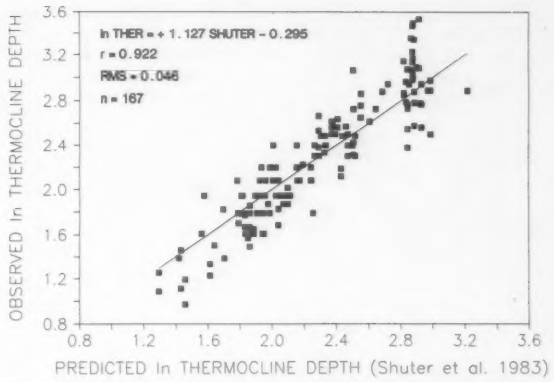
ln THER = + 1.127 SHUTER - 0.295
r = 0.922
RMS = 0.046
n = 167
OBSERVED ln THERMOCLINE DEPTH
PREDICTED ln THERMOCLINE DEPTH (Shuter et al. 1983)

FIG. 2. Relationship between observed thermocline depth (m) and the predictions from the model of Shuter et al. (1983). Both axes are on a natural logarithmic scale.

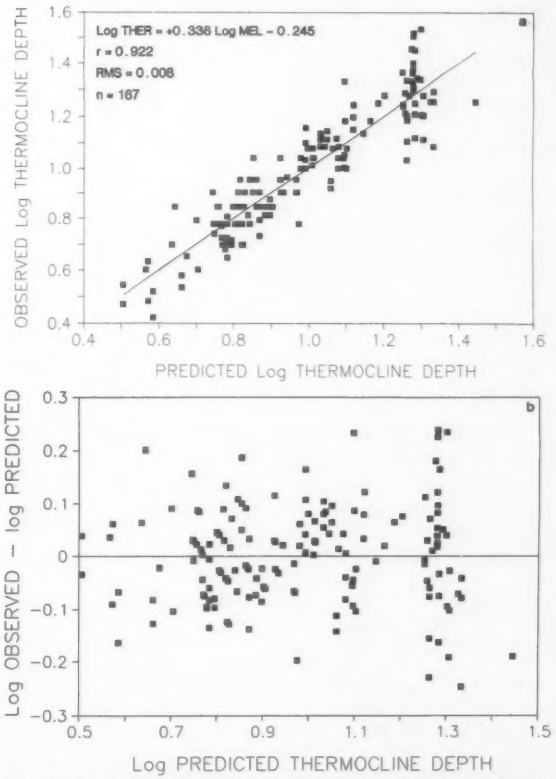

FIG. 3. (a) Relationship between observed thermocline depth and *MEL* predicted thermocline depth by the model generated in this study. Both axes are on a decimal logarithmic scale. (b) Plot of the residuals of the relationship in Fig. 3a, between the decimal logarithms of observed thermocline depth and predicted thermocline depth, according to the model generated in this study.

TABLE 7. Alternate regression equations for predicting thermocline depth for the data set studied ($n = 167$). All coefficients are significantly ($p < 0.05$) different from zero. Listed are the correlation coefficient (r), and the residual mean square (*RMS*).

Equation	r	*RMS*
(2) Log *THER* = 0.185 Log (A) + 0.842	0.91	0.009
(3) Log *THER* = 0.282 Log (L) − 0.225	0.91	0.010

than that of Shuter et al. (1983). These differences are to be expected because this model is fit to these data. However, because these data are more extensive and more varied, it is likely that the model presented here is also a better tool for predicting mixing depth in other lakes for it is more representative of the average relationship for temperate lakes.

Conclusion

The departures of the predictions based on the 17 published models from those of the model formulated in this study (equation (1)) might reflect both the regional character of the different models, and the limited ranges of their predictive variables. Even though the model of Shuter et al. (1983) is biased, it remains the best published one available for predicting mixing depth. However, since the relationship reported here is based on a more extensive data set ($n = 167$), covering a wider range of lake sizes than that of Shuter et al. (1983), it should be more representative of the average relationship for temperate lakes.

The results show that the depth of the thermocline at maximum heat content depends primarily upon *MEL*, since most of the observed variation in thermocline depth is accounted for by this variable. Thermocline depth is also correlated, but to a slightly lesser extent with *ML*, *MW*, *MEW*, A, L, D_L, Z_{max}, Z_{avg}, Z_{rel} and V. Factors such as latitude or altitude did not explain any of the residual variance.

Within the limits of this study, the results suggest that the model using log *MEL* (equation (1)) is the most effective predictor of mixing depth of temperate lakes with *MEL* of up to 40 km. Two other models using area and length of shoreline have been proposed as alternate predictive tools, if *MEL* is not readily available.

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A Multivariate Maturity Model with a Comparative Analysis of Three Lingcod (*Ophiodon elongatus*) Stocks

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In this paper we develop a multivariate analysis of length and age at maturity that includes the univariate maturity model of Schnute and Richards (1990, Can. J. Fish. Aquat. Sci. 47: 24–40) as a special case. In addition, we address the problem of drawing meaningful conclusions from large data sets oriented to fish maturity, and we present statistical tests of such conclusions. We illustrate our approach with comparisons among male and female lingcod (*Ophiodon elongatus*) from three stocks along the coast of British Columbia, Canada. From the univariate analysis, we demonstrate that male lingcod mature at a smaller size than female lingcod, and that for each sex, size at maturity increases with latitude. From the multivariate analysis, we determine that length and age together provide a better prediction of lingcod maturation than either variate considered alone. The multivariate model is applicable to any situation for which one or more positive variates is asymptotically related to a probability measure in the range (0,1).

Dans cette étude, les auteurs ont mis au point une analyse multifactorielle de la longueur et de l'âge à maturité qui inclut le modèle de maturité unifactoriel de Schnute et Richards (1990, J. can. sci. halieut. aquat. 47 : 24–40) à titre de cas spécial. En outre, ils traitent du problème que pose la dérivation de conclusions significatives à partir de gros ensembles de données orientés en fonction de la maturité du poisson et présentent des tests statistiques pour ces conclusions. Ils illustrent leur approche par des comparaisons entre des ophiodons (*Ophiodon elongatus*) mâles et femelles provenant de trois stocks le long de la côte de la Colombie-Britannique au Canada. D'après l'analyse unifactorielle, les auteurs démontrent que l'ophiodon mâle atteint la maturité à une taille plus petite que la femelle, et que pour chaque sexe, la taille à maturité augmente avec la latitude. D'après l'analyse multifactorielle, ils déterminent que la longueur et l'âge permettent, ensemble, de faire une meilleure prévision de la maturation de l'ophiodon que l'une ou l'autre de ces variables considérées seules. Le modèle multifactoriel peut être appliqué à toute situation dans laquelle une ou plusieurs variables positives sont asymptotiquement reliées à une mesure de probabilité de l'ordre (0,1).

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In this paper we present an analysis of length and age at maturity for three lingcod (*Ophiodon elongatus*) stocks off the coast of British Columbia, Canada. We emphasize the importance of two phases in the analysis: an exploratory phase for hypothesis formulation and a modeling phase for parameter estimation and hypothesis testing. More generally, we address the problem of drawing meaningful conclusions from large biological data sets oriented to fish maturity. As an aid to hypothesis testing, we develop a multivariate maturity model that accounts for multiple attributes associated with maturity information.

Fisheries sample data sets usually consist of records of individual fish taken from catches that are categorized, for example, according to gear type, locality, month, and year. The first step in our exploratory analysis is to determine the categories of information that reflect meaningful changes in maturity relationships. We employ tabular histograms (Jensen et al. 1986) to facilitate comparisons that lead to hypothesis formulation. For example, we hypothesize that there are greater among-area differences in length at 20% than at 80% maturity for female lingcod.

Our preliminary analysis suggests that both length and age contain information on lingcod maturity. Hence, to account for both attributes simultaneously, we require a multivariate approach. We develop a multivariate maturity model that generalizes the Schnute-Richards (1990) univariate model and thus encompasses a wide range of historical models relating to growth, maturity, and survivorship. Furthermore, our model can be formulated in terms that are similar to the generalized linear model (McCullagh and Nelder 1983) and the general dose–response model of Schnute and Jensen (1986). In particular, for a specified percentage maturity, our formulation leads to estimates of length–age contours analogous to Schnute-Jensen dose–response curves. Consequently, in the multivariate framework, length at 50% maturity is a function of age, rather than a fixed parameter. This feature distinguishes our work from other recent maturity analyses (Welch and Foucher 1988; Schnute and Richards 1990).

One method for analyzing multivariate maturity data might be to transform the data appropriately and then conduct a standard multiple regression on the transformed data. For example, one might apply a logit transform to percent maturity and a log or square root transform to the age and length data. There are two problems with this approach. First, as discussed by Welch and Foucher (1988) in the context of univariate data, the least squares likelihood fails to account for the binomial nature of maturity data. Second, appropriate data transforms may not be

obvious. Arbitrary choices, such as logit, log, or square root may impose unnecessary constraints on the analysis.

The first problem can be easily circumvented by using the generalized linear model (McCullagh and Nelder 1983), rather than the classical regression model. This allows a much broader choice of model error structure, including binomial error. However, the generalized linear model requires the data to be transformed in advance, and thus does not resolve the second problem cited above. At best, an analysis might involve trying several transforms and arbitrarily choosing the one deemed most suitable. By contrast, a nonlinear model permits the analysis to determine the transforms, and that is the approach we take here.

In our model, transform parameters are incorporated as part of the model's parameter set. This provides considerable flexibility in curve shape and allows the model to embrace a wide range of data sets. Furthermore, confidence intervals and joint confidence regions for the transform parameters, as well as other model parameters, can be obtained directly from the likelihood function. Such confidence regions can be used to identify statistical differences among maturity relationships from samples of various populations.

In section 1 of the paper, we present the multivariate model, along with a complete statistical theory for obtaining parameter estimates and confidence regions. In section 2, we describe data sources for our analysis, produce tabular histograms of the data, and propose various preliminary hypotheses. Finally, in section 3, we apply the model to test hypotheses formulated in section 2 and thus to distinguish populations with different maturity relationships. The example shows that our methods are widely applicable to comparative, multivariate maturity analysis.

1. The Model

As stated in the introduction, our model relates to the models of McCullagh and Nelder (1983), Schnute and Jensen (1986), and Schnute and Richards (1990). Although we could begin this section by motivating our model from these earlier models, we choose a more straightforward approach. We first give a detailed description of the multivariate model and then demonstrate its connections with earlier work. See Schnute and Richards (1990) for a more complete historical approach to the model's univariate version.

The multivariate model describes the response y, with $0 \leq y \leq 1$, to a vector $\mathbf{x} = (x_1,..,x_k)$ of k positive attributes. For example, when $k = 2$, y could be the probability of maturity for fish with length x_1 and age x_2. Our description of the function $y(\mathbf{x})$ involves a transform ξ_i of each x_i, as well as a transform η of y. The function $y(\mathbf{x})$ can be constructed from the three equations

$$(1.1) \quad \xi_i = \frac{x_i^{c_i} - u_i^{c_i}}{U_i^{c_i} - u_i^{c_i}},$$

$$(1.2) \quad \eta = \alpha + \sum_{i=1}^{k} \gamma_i \xi_i,$$

$$(1.3) \quad \eta = \ln\left[\frac{v^{-b}-1}{y^{-b}-1}\right] / \ln\left[\frac{v^{-b}-1}{V^{-b}-1}\right],$$

subject to the constraints

$$(1.4a) \quad 0 < v < V < 1,$$

$$(1.4b) \quad 0 < u_i < U_i;\ i = 1,..,k.$$

To understand how equations (1.1)–(1.3) define $y(\mathbf{x})$, notice first that they involve the variables $\mathbf{x}$, $\boldsymbol{\xi} = (\xi_1,..,\xi_k)$, η, and y, as well as $4k+4$ constants: the 4 scalars

$$(1.5a) \quad b,\ \alpha,\ v,\ V,$$

and the $4k$ components of the vectors

$$(1.5b) \quad \mathbf{c},\ \boldsymbol{\gamma},\ \mathbf{u},\ \mathbf{U},$$

where $\mathbf{c} = (c_1,..,c_k)$, $\boldsymbol{\gamma} = (\gamma_1,..,\gamma_k)$, $\mathbf{u} = (u_1,..,u_k)$, and $\mathbf{U} = (U_1,..,U_k)$. Suppose that the constants (1.5) are all known. The transform (1.1) then defines the connection between the variable x_i and the corresponding variable ξ_i. Next, the relation (1.2) expresses η as a linear combination of the ξ_i. Finally, (1.3) determines the connection between η and y. Thus, if $\mathbf{x}$ is given, (1.1) determines $\boldsymbol{\xi}$ and (1.2) determines η. The response y, in turn, may be obtained from η by the back-transform of (1.3):

$$(1.6) \quad y = [1 + (v^{-b} - 1)e^{-\beta\eta}]^{-1/b},$$

where

$$(1.7) \quad \beta = \ln\left[\frac{v^{-b}-1}{V^{-b}-1}\right].$$

We include the constant β in (1.6)–(1.7) for consistency with Schnute and Richards (1990).

In (1.1)–(1.3), the $2k+2$ constants $(\mathbf{u},\mathbf{U},v,V)$ are fixed by the investigator prior to the analysis, in accord with the ranges of available data. Values of u_i and U_i are selected to represent typical low and high values of x_i, respectively. Similarly, v and V are fixed at typical low and high values of y. The choice of these constants will affect estimates of the coefficients α and $\boldsymbol{\gamma}$ in (1.2), but not shape of the maturity relationship or the estimates and confidence intervals for the exponents b and $\mathbf{c}$. The constants $(\mathbf{u},\mathbf{U},v,V)$ act simply to scale the data, so that the transformed variables $\boldsymbol{\xi}$ and η lie, for the most part, in the range (0,1). Since these transformed variables are linearly related by (1.2), the coefficients α and $\boldsymbol{\gamma}$ typically have magnitudes comparable to 1.

The constraints (1.4) on the constants $(\mathbf{u},\mathbf{U},v,V)$ ensure that each ξ_i is an increasing function of x_i and that y is an increasing function of η. The model assumes that each x_i has a monotone influence on y. The sign of γ_i determines whether y increases ($\gamma_i > 0$) or decreases ($\gamma_i < 0$) as x_i increases. The reader can verify that ξ_i varies from 0 to 1 as x_i ranges from u_i to U_i; similarly, η goes from 0 to 1 as y increases from v to V.

With this background, we can now demonstrate how the model (1.1)–(1.4) relates to earlier models. First, if the exponents $(b,\mathbf{c})$ are considered known, then our model is a special case of the generalized linear model (McCullagh and Nelder 1983) with a linear predictor η in (1.2) and a link function (1.3) between η and y. When $b = 1$, the right side of (1.3) is a linear function of $\ln[y/(1-y)]$, i.e. the classical logit. Thus, (1.3) generalizes the logit link function (McCullagh and Nelder 1983, p. 23), which is the canonical link for binomial data.

Second, the model (1.1)–(1.4) is analogous to the multivariate dose–response model of Schnute and Jensen (1986). Although they consider primarily a real response y in the range $(0,\infty)$, they include a proposal (their equation (1.15)) for modeling data in which y is restricted to (0,1). Our transform (1.3) is tailored after their suggestion, except that η here is scaled to the range (0,1), rather than (−1,1). When $k = 2$, their analysis of curve types (their figures 1–3) precisely describes the various possible shapes for model contours on which $y(x_1,x_2)$ is constant. We illustrate this point in an example below.

Third, the model (1.1)–(1.4) is a simple extension of the univariate model of Schnute and Richards (1990). When $k=1$, our model involves the eight constants b, c_1, α, γ_1, u_1, U_1, v, and V. Similarly, the Schnute-Richards model includes the six constants b, c, x_1, x_2, y_1, y_2. The two models are identical if we relate our constants to theirs as shown below:

$$\begin{array}{cccccccc} b & c_1 & \alpha & \gamma_1 & u_1 & U_1 & v & V \\ | & | & | & | & | & | & | & | \\ b & c & 0 & 1 & x_1 & x_2 & y_1 & y_2 \end{array}$$

To make the comparison complete, we must also identify our ξ_1 with their ξ and our η with their η/β. Both models ultimately involve only four nonredundant parameters. Here, we regard (u_1,U_1,v,V) as fixed and treat (b,c_1,α,γ_1) as unknown parameters. Alternatively, we could set $(\alpha,\gamma_1)=(0,1)$, regard (v,V) as fixed, and treat (b,c_1,u_1,U_1) as unknown parameters. This latter point of view corresponds to that of Schnute and Richards (1990).

When $k=1$, the parameters u_1 and U_1 are particularly meaningful in the context of maturity data. For example, as discussed by Schnute and Richards (1990), u_1 and U_1 could represent size at 50% and 80% maturity, respectively, with $(v,V)=(0.5,0.8)$. When $k>1$, however, this concept no longer applies. For example, suppose that $k=2$ and that maturity y is related to both size x_1 and age x_2. Then the condition

$$y(x_1,x_2) = 0.5$$

determines a curve along which size at 50% maturity depends on age. For this reason, in contrast with Schnute and Richards (1990), we adopt α and γ as parameters for the multivariate model and retain the constants $(\mathbf{u},\mathbf{U},v,V)$ only as scaling factors.

In summary, our discussion so far has focused on representing y as a function of the k-dimensional attribute vector $\mathbf{x}$ and the parameter vector

$$\text{(1.8a)}\quad \boldsymbol{\theta} = (\alpha,\boldsymbol{\gamma},b,\mathbf{c})$$

with $2k+2$ components. The vector

$$\text{(1.8b)}\quad \boldsymbol{\psi} = (\mathbf{u},\mathbf{U},v,V)$$

of the remaining $2k+2$ constants (1.5) is prescribed by the investigator, where the components of $\boldsymbol{\psi}$ are used to scale the data. In some cases the components of $\boldsymbol{\theta}$ may also be restricted, as discussed in the context of hypothesis testing below.

To apply the model, we must relate $y(\mathbf{x},\boldsymbol{\theta})$ to probability theory and determine a corresponding likelihood function. In most analyses, attribute data are combined over intervals or cells. For example, let $\mathbf{x}_j=(x_{1j},..,x_{kj})$, $j=1,..,n$, represent the attribute vector at the centre of the jth cell. Within each cell j, suppose that a total of N_j fish are sampled, of which M_j are mature. Thus, the data consist of triplets $(\mathbf{x}_j,M_j,N_j)$ with $j=1,..,n$. If each M_j is binomially distributed with probability $y(\mathbf{x}_j,\boldsymbol{\theta})$ among the N_j trials, then the likelihood function for these data is the product

$$\text{(1.9)}\quad L(\boldsymbol{\theta}) = \prod_{j=1}^{n} \left\{ \binom{N_j}{M_j} y(\mathbf{x}_j,\boldsymbol{\theta})^{M_j} \, [1-y(\mathbf{x}_j,\boldsymbol{\theta})]^{N_j-M_j} \right\} ,$$

where the notation $\binom{N}{M}$ denotes a binomial coefficient.

The usual derivation of the likelihood function (1.9) involves the assumption that each M_j is conditional on a specified sample size N_j. In practice, however, it often turns out that N_j is determined by the sample distribution of the $\mathbf{x}_j$. Even in this case (1.9) remains valid, as long as N_j is not conditional on $\boldsymbol{\theta}$. When N_j depends on $\mathbf{x}_j$, then an additional factor could be added to (1.9) to include parameters related to the distribution of $\mathbf{x}_j$. Such an extension is beyond the scope of this paper.

It follows from (1.9) that twice the negative log likelihood, except for a constant independent of $\boldsymbol{\theta}$, is given by

$$\text{(1.10)}\quad l(\boldsymbol{\theta}) = -2 \sum_{j=1}^{n} \left\{ \begin{array}{l} M_j \ln[y(\mathbf{x}_j,\boldsymbol{\theta})] \\ + \, (N_j-M_j) \ln[1-y(\mathbf{x}_j,\boldsymbol{\theta})] \end{array} \right\} .$$

Note that $l(\boldsymbol{\theta})\geqslant 0$, since $0<y<1$ and $0\leqslant M_j\leqslant N_j$. The minimum of $l(\boldsymbol{\theta})$ determines the maximum likelihood parameter estimate $\hat{\boldsymbol{\theta}}$. We obtain $\hat{\boldsymbol{\theta}}$, as required in section 3 below, by the simplex search method (Nelder and Mead 1965; Press et al. 1986, p. 289–293), as implemented by Mittertreiner and Schnute (1985).

The likelihood function $l(\boldsymbol{\theta})$ can also be used to determine approximate confidence regions for the model parameters. To be precise, suppose that $\boldsymbol{\theta}$ has dimension r and that $\boldsymbol{\theta}_1$ is an s-dimensional subset of $\boldsymbol{\theta}$. Let $\boldsymbol{\theta}_2$ denote the remaining set of $r-s$ parameters, so that $\boldsymbol{\theta}=(\boldsymbol{\theta}_1,\boldsymbol{\theta}_2)$. Define

$$\text{(1.11)}\quad l_1(\boldsymbol{\theta}_1) = \min_{\boldsymbol{\theta}_2} l(\boldsymbol{\theta}_1,\boldsymbol{\theta}_2) ;$$

that is, for a given $\boldsymbol{\theta}_1$, let $l_1(\boldsymbol{\theta}_1)$ represent the minimum of $l(\boldsymbol{\theta})$ with respect to $\boldsymbol{\theta}_2$. Then, for $0<p<1$, the boundary of a p-level confidence region for $\boldsymbol{\theta}_1$ is determined by values of $\boldsymbol{\theta}_1$ that solve the equation

$$\text{(1.12)}\quad l_1(\boldsymbol{\theta}_1) = l(\hat{\boldsymbol{\theta}}) + \chi^2(s,p),$$

where $\chi^2(s,p)$ represents the cutoff at level p for the χ^2 distribution with s df. This region is approximate, based on the likelihood ratio test for large sample sizes (Kendall and Stuart 1979, chap. 24).

Solving for p in (1.12) gives

$$\text{(1.13)}\quad p = (\chi^2)^{-1} \, [s, l_1(\boldsymbol{\theta}_1) - l(\hat{\boldsymbol{\theta}})],$$

where $(\chi^2)^{-1}$ denotes the inverse of the function $\chi^2(s,\cdot)$ in (1.12). The expression (1.13) gives an approximate p-level for rejecting a proposed value of $\boldsymbol{\theta}_1$. When $s=1$ (so that $\boldsymbol{\theta}_1$ is actually a scalar θ_1), the graph of p versus θ_1 from (1.13) is called a probability profile for θ_1. Typically, this profile is a V-shaped curve centered at $\hat{\theta}_1$.

The criterion (1.12) can also be used to resolve an important issue in the multivariate context: whether or not model fit is significantly improved by adding an extra factor. As the number of factors increases, $\boldsymbol{\theta}$ includes more parameters and the minimum $l(\hat{\boldsymbol{\theta}})$ decreases. Statistically, (1.12) allows us to assess whether or not the df increase is justified by an adequate reduction in $l(\hat{\boldsymbol{\theta}})$.

We illustrate this process when $k=2$, since this case has special interest in the examples below. The parameter vector (1.8a) is then equivalent to

$$\text{(1.14)}\quad \boldsymbol{\theta} = (\alpha,\gamma_1,\gamma_2,b,c,d) ,$$

where $c_1=c$ and $c_2=c+d$. Here, we use d to denote the difference c_2-c_1 between the exponents associated with the two factors. Let H_A denote the hypothesis that the six parameters (1.14) are unrestricted. An interesting restriction to H_A is the hypothesis

$$\text{(1.15)}\quad H_B\colon d=0.$$

TABLE 1. Five possible tests associated with hypotheses (1.15)–(1.17). In each case, the null (H_0) and alternative (H_1) hypotheses are indicated, along with the associated df. Components of the parameter vector $\boldsymbol{\theta}=(\boldsymbol{\theta}_1,\boldsymbol{\theta}_2)$ are also shown; both H_0 and H_1 leave $\boldsymbol{\theta}_2$ unrestricted, but H_0 assumes that $\boldsymbol{\theta}_1=0$. In two cases, both H_0 and H_1 involve the restriction $d=0$.

H_0	H_1	df	$\boldsymbol{\theta}_1$	$\boldsymbol{\theta}_2$	Restriction
H_C	H_B	1	(γ_1)	(α,γ_2,b,c)	$d=0$
H_D	H_B	1	(γ_2)	(α,γ_1,b,c)	$d=0$
H_B	H_A	1	(d)	$(\alpha,\gamma_1,\gamma_2,b,c)$	
H_C	H_A	2	(γ_1,d)	(α,γ_2,b,c)	
H_D	H_A	2	(γ_2,d)	(α,γ_1,b,c)	

When H_B is true, the exponents are equal: $c_1=c_2=c$. Two further restrictions involve the hypotheses that one of the two factors is missing, that is,

(1.16) H_C: $d=0$, $\gamma_1=0$,

(1.17) H_D: $d=0$, $\gamma_2=0$.

Each hypothesis (1.15)–(1.17) can be used as a null hypothesis against the alternative H_A. Since two parameters (γ_i,c_i) automatically drop out when one factor is removed, the hypothesis H_B provides a convenient intermediary between H_A and either H_C or H_D.

The analysis of Schnute and Jensen (1986, figures 1–3) provides some insight into the model restricted by H_B. Assume, as in our examples below, that y is an increasing function of both x_1 and x_2. Then the parameter c determines the shape of contours along which $y(x_1,x_2)$ is constant. When $c>1$, the contours are convex down, intersecting the x_1 and x_2 axes (Schnute and Jensen 1986, figures 1 and 3, type 16). When $c=1$, the curves are linear. When $0<c<1$, the contours are convex up, intersecting both axes (type 9b). Finally, when $c<0$, the contours are convex up, but asymptotic to both axes (type 15).

The four hypotheses H_A–H_D suggest up to five possible tests with specified null (H_0) and alternative (H_1) hypotheses. Table 1 relates each test to (1.12), where the null hypothesis for the parameter vector $\boldsymbol{\theta}=(\boldsymbol{\theta}_1,\boldsymbol{\theta}_2)$ is

$$H_0\colon\ \boldsymbol{\theta}_1 = 0\ .$$

A variant on this scheme might involve further restrictions on $\boldsymbol{\theta}$. For example, we might fix $b=1$ in H_A–H_D to restrict the model to the canonical logit link.

In analogy with stepwise regression (e.g. Draper and Smith 1981, chap. 6), the following 3-step procedure might be used to build a maturity model with $k\leq 2$. First, choose the best univariate case, H_C or H_D, as described above. Second, if possible, reject the best univariate model in favor of a bivariate model with H_B. Adopt a univariate model unless H_B is justified. Third, if a bivariate model is indicated in step 2, then test H_B against H_A. The outcome of this test decides whether or not to assume that $d=0$ in the bivariate model. This procedure should, however, be regarded somewhat informally, since other reasonable testing sequences are possible. For example, notice that only the first three tests in Table 1 are used. An alternative approach might be to consider p-levels associated with all five tests as Bayesian measures of relative model credibility.

It should be emphasized that estimates $\hat{\boldsymbol{\theta}}$ from (1.10) reflect properties of the sampled population. Typically, this is the commercial catch, which may or may not have properties different from those of the underlying fish population. For example, suppose that the fishery is size-selective, but within a size-class, not selective for maturity. Then estimates $\hat{\boldsymbol{\theta}}$ are representative of the underlying fish population if size is among the x_i. By contrast, a univariate analysis with age as the only attribute might lead to estimates $\hat{\boldsymbol{\theta}}$ biased relative to $\boldsymbol{\theta}$ for the fish population. This could occur if the fishery selects for larger fish within a given age class and if larger fish are more likely to be mature.

2. Exploratory Analysis

We illustrate our approach with data from three lingcod stocks off British Columbia, Canada, in Pacific Marine Fisheries Commission (PMFC) Major Areas 3C-N, 5A, and 5B (Fig. 1). Commercial trawl fisheries account for most of the lingcod catch from these areas. Biological samples including maturity and age data have been collected since 1977 (e.g. Cass et al. 1988). In most instances, a biological sample represents approximately 200 fish, selected randomly from the catch. Within each sample, all fish are measured for length. Furthermore, some samples are chosen for age determination, and within these, all fish are aged. Hence, as discussed in section 1, the N_j are determined by the distribution of the $\mathbf{x}_j$.

Lingcod age is obtained from fin rays, following a method (Beamish and Chilton 1977) which has been partially validated for the Area 3C-N stock (Cass and Beamish 1983; Schnute et al. 1989). Fork length has been measured to the nearest centimetre, or nearest millimetre for a few later samples. Methods of recording maturity have varied since 1977, with respect to the number of subgroups noted for immature or mature fish. We disregard these subgroups and consider fish to be either immature or mature.

In 1985, data on lingcod maturity were collected during a research cruise to Areas 3C-N and 5B (Cass et al. 1986). Cruise results on the maturity of female lingcod are consistent with commercial samples from the same time period, and the data sets have been combined. However, this was not true for male lingcod, and we have not included the male cruise data in the analysis. Most male lingcod caught during the cruise were coded as mature, although later histological examination indicated at least some of these fish to be immature.

Once the basic data have been assembled, it is important to consider the number of data categories that can be meaningfully examined. Here, we regard length and age as continuous attributes with an asymptotic relationship to maturity. This relationship may depend on the discrete categories of sex, area, year, and month of collection. Table 2 illustrates the distribution of samples according to these categories. Each entry in the table represents a sample size that is scaled relative to the maximum sample size in any category. An underscore indicates that age was determined for the sample.

Several points are evident from the sample distributions in Table 2. First, samples are not available for each year in each area. In particular, Area 5A is represented by relatively few samples, especially for lingcod age. Second, there has been a distinctly seasonal component to data collection. Most samples have been obtained between May and November, a period that excludes winter spawning. For this reason, we include only May to November samples in all analyses below. Third, male lingcod maturity data are represented by fewer samples than female lingcod maturity data, and for samples that include both sexes, the sample size is usually larger for female lingcod. These sample size biases reflect a female bias in the lingcod

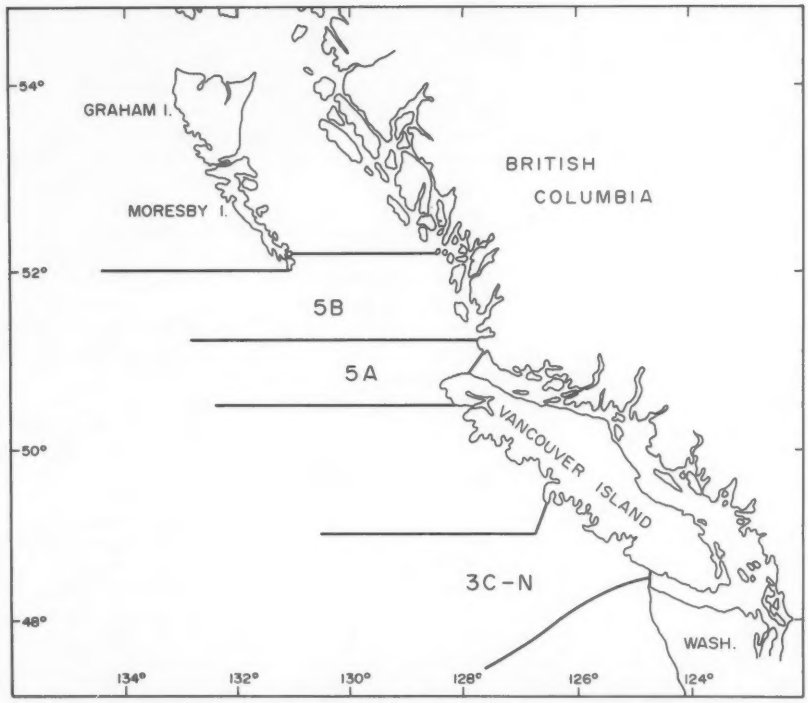

FIG. 1. Coast of British Columbia, Canada, showing Areas 3C-N, 5A, and 5B.

trawl catch. At certain times, male lingcod appear to be unavailable to the trawl gear. Hence, the biological implications of the analysis must be interpreted cautiously for male lingcod.

Given the sample distribution, a reasonable next step is to examine annual variation in the maturity–length relationship by area and sex, combining sample data by month. Tabular histograms for the female maturity–length relationship are presented in Table 3, along with corresponding tabular histograms for the sample frequencies. It appears that the relationship is to some extent annually variable for each area, and that the annual trends may or may not be consistent among areas. In general, the size at which maturation begins tends to be more variable than the size at which maturation is complete. Similar patterns are evident in the maturity–length relationship for male lingcod (not shown). As there are no years that appear obviously biased, after accounting for sample size structure, we group sample data over years for the remaining analyses.

Tabular histograms for the maturity–length relationship are presented by area and sex in Table 4. It appears that the relationship is dependent on both sex and area. For example, male lingcod generally mature at a smaller size than female lingcod. The pattern among areas is more complex. For each sex, maturation is complete at a similar size in each area, although maturation begins at different sizes. Note from the sample frequency histograms that the majority of fish sampled have large sizes. Because of this sample distribution, there is greater uncertainty in the size at which maturation begins than in the size at which maturation is complete. This becomes evident in the statistical analysis in section 3.

Female lingcod tend to be larger than male lingcod at a given age. Hence, tabular histograms for the maturity–age relationship (Table 5) differ somewhat from those for the maturity–length relationship. Male lingcod may begin maturation at either a younger (Area 3C-N) or older (Area 5B) age than female lingcod from the same area. For both sexes, fish from Area 3C-N mature at a younger age than fish from Area 5B. Too few age data are available from Area 5A to draw any meaningful conclusions, and Area 5A is excluded from further analyses that include age.

Based on Tables 4 and 5, both length and age appear to be useful maturity attributes for most cases. We next examine to what extent a knowledge of age improves the prediction of maturation, given length. This is illustrated by tabular histograms in Table 6. Each column of the table represents the maturity–age relationship for a given length, sex, and area. Within the length range over which maturation occurs, there is a general tendency for the percentage mature to increase in each column with increasing age. As in Tables 4 and 5, the maturity relationships appear to be sex and area specific. Thus, it appears that length and age together provide a better prediction of maturation than either attribute alone. This conclusion will be evaluated further in section 3.

3. Statistical Analysis

Our exploratory analysis suggests that lingcod maturity–length relationships depend on sex and area. Here, we demonstrate that differences in these relationships are, indeed, real statistically. We then perform similar analyses for the maturity–age relationship. Finally, we examine the joint effects of length and age on lingcod maturation using the multivariate model.

Parameter estimates for lingcod maturity–length relationships (Table 7) are obtained by sex and area from (1.10), fol-

TABLE 2. Tabular histograms of the number of lingcod sampled for length and maturity status each month by sex, area, and year. The symbol for each histogram cell represents the sample size on a scale of 0 to 10, relative to a maximum of 416 female lingcod sampled from Area 3C-N in August 1985. For emphasis, '*' indicates '10', '·' indicates an absence of data, and an underscore indicates samples for which lingcod age has been determined.

		Female samples by month												Male samples by month											
Area	Year	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
3C	77	4	2	5	1	·	·	5	·	·	·	5	·	1	1	1	0	·	·	2	·	·	·	0	·
3C	78	·	·	·	·	·	·	·	·	1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
3C	79	·	·	·	·	2	·	·	·	·	·	2	·	·	·	·	·	5	·	·	·	·	·	0	·
3C	80	·	·	·	·	·	1	0	·	·	·	·	·	·	·	·	·	·	1	1	·	·	·	·	·
3C	81	·	·	·	·	·	·	·	·	1	·	·	·	·	·	·	·	·	·	·	·	3	·	·	·
3C	82	·	·	·	·	·	6	3	·	·	·	·	·	·	·	·	·	·	3	3	·	·	·	·	·
3C	83	·	·	·	·	·	4	3	·	·	·	·	·	·	·	·	·	·	2	3	·	·	·	·	·
3C	85	·	·	·	·	8	·	3	*	3	2	·	·	·	·	·	·	3	·	1	0	2	1	·	·
3C	86	·	·	·	·	·	3	·	·	·	·	·	·	·	·	·	·	·	1	·	·	·	·	·	·
3C	88	·	·	·	·	·	·	·	4	4	1	·	·	·	·	·	·	·	·	·	2	3	0	·	·
5A	77	·	·	·	·	1	·	·	·	·	·	·	·	·	·	·	·	0	·	·	·	·	·	·	·
5A	78	5	·	·	·	·	·	·	·	·	1	0	1	·	·	·	·	·	·	·	·	·	·	·	·
5A	81	·	·	·	·	·	·	·	·	2	·	·	·	·	·	·	·	·	·	·	·	3	·	·	·
5A	83	·	·	·	·	·	4	·	·	·	·	·	·	·	·	·	·	·	3	·	·	·	·	·	·
5A	85	·	·	·	·	·	·	·	·	8	·	·	·	·	·	·	·	·	·	·	·	3	·	·	·
5A	88	·	·	·	·	·	·	2	·	·	·	·	·	·	·	·	·	·	·	2	·	·	·	·	·
5B	77	·	·	·	·	·	6	·	·	·	·	·	·	·	·	·	·	·	2	·	·	·	·	·	·
5B	78	·	·	·	·	·	·	·	1	·	·	·	·	·	·	·	·	·	·	·	1	·	·	·	·
5B	79	·	·	·	·	·	·	·	2	4	·	·	·	·	·	·	·	·	·	·	1	0	·	·	·
5B	80	·	·	·	·	·	2	3	·	·	·	·	·	·	·	·	·	·	3	4	·	·	·	·	·
5B	81	·	·	·	·	·	·	·	·	3	·	·	·	·	·	·	·	·	·	·	·	1	·	·	·
5B	82	·	·	·	·	·	7	·	·	·	3	·	·	·	·	·	·	·	3	·	·	·	1	·	·
5B	83	·	·	·	·	6	4	·	·	·	·	·	·	·	·	·	·	1	2	·	·	·	·	·	·
5B	85	·	·	·	·	·	4	·	5	1	3	·	·	·	·	·	·	·	1	·	·	·	0	·	·
5B	88	·	·	·	·	·	4	·	·	·	·	·	·	·	·	·	·	·	0	·	·	·	·	·	·

lowing the methods described in section 1. Sample data must be grouped into length intervals for the analysis, and we choose 1-cm groupings. In addition, application of the model (1.1)–(1.4) requires that certain quantities be prescribed. For the univariate application with $k=1$, we set $(\alpha,\gamma_1)=(0,1)$, regard (v,V) as fixed, and treat (b,c_1,u_1,U_1) as unknown parameters. We select $(v,V)=(0.2,0.8)$, so that u_1 and U_1 correspond to sizes at 20% and 80% maturity, respectively. Thus, u_1 represents a size near which maturation begins and U_2 a size near complete maturation. The analysis in section 2 indicates that these sizes are particularly meaningful for the lingcod example. For comparison, we also evaluate size at 50% maturity ($y=0.5$) for each set of parameter estimates.

Schnute and Richards (1990) note that one of the exponents (b or c_1) may be redundant. To test this possibility, we include parameter estimates and minimum values $l(\boldsymbol{\theta})$ for fixed $b=1$ and for b unrestricted in Table 7. The choice $b=1$ is an obvious one, as it corresponds to the canonical link function. Values of $l(\boldsymbol{\theta})$ for $b=1$ in Table 7 differ by only a small amount from the corresponding minimum value with b unrestricted. By (1.12), we cannot reject the hypothesis that $b=1$. We assume $b=1$ in tests of other parameters below.

Parameter estimates in Table 7 are consistent with results from exploratory analysis. For both male and female lingcod, there are greater area differences in size at 20% maturity, than in size at 50% or 80% maturity. In each area, male lingcod are 80% mature at a size smaller than that at which female lingcod are 50% mature. Predicted maturity relationships are shown in Fig. 2. It is evident from the figure that male lingcod have a consistently higher percentage maturity for a given size than female lingcod. In addition, male lingcod mature at progressively larger sizes from Area 3C-N to Area 5A, and Area 5B. Since this area progression corresponds to a northerly movement (Fig. 1), size at maturity for male lingcod appears to increase with latitude. Female lingcod appear to exhibit a similar latitudinal pattern in size at maturity, although the effect is less pronounced.

As discussed by Schnute and Richards (1990), the parameter c_1 corresponds to a shape parameter. The estimates of c_1 in Table 7 vary considerably, and probability profiles for c_1 in Fig. 3 indicate that this variability is real statistically. For example, the 95% confidence interval for c_1 obtained for male lingcod from Area 3C-N does not overlap with 95% confidence intervals for female lingcod from Area 3C-N or 5B. These probability profiles are useful for pairwise comparisons and for suggesting the results of multiple comparisons. More rigorous tests of multiple comparisons are unnecessary for our argument here. However, they are possible by simple extensions of our techniques. Consider, for example, the hypothesis that c_1 differs between sexes. In this case, one would use a joint likelihood function for both data sets obtained from the product of the likelihoods for each. The number of parameters would depend on whether or not the c_1-values were equal, and a test based on (1.12) would apply.

Corresponding to the choice $(v,V)=(0.2,0.8)$, we can obtain probability profiles and joint confidence regions for sizes near the start and end of the maturation process. Probability profiles for size at 20% maturity are shown in Fig. 4. The profiles fur-

TABLE 3. Tabular histograms of percentage maturity at length (cm) for female lingcod by area and year. Sample sizes (N) and corresponding sample frequency histograms are also shown. The symbol for each histogram cell represents the cell value (percentage mature or sample frequency) on a scale of 0 to 10, where '*' indicates '10' and '·' indicates an absence of data. Data for each year are grouped into 5-cm length intervals. The first interval includes data for lengths <50 cm, and the last interval includes data for lengths ≥90 cm.

		Percent mature at length										Frequency at length									
Area	Year	50		60		70		80		90+	N	50		60		70		80		90+	
3C	77	0	·	0	7	9	*	*	*	*	*	192	0	·	0	1	2	2	3	*	7	5
3C	78	·	·	·	·	0	*	*	*	*	*	47	·	·	·	·	0	2	2	*	*	*
3C	79	·	·	·	·	*	*	*	*	*	*	82	·	·	·	·	1	2	6	8	*	9
3C	80	·	·	0	0	0	7	9	*	*	*	82	·	·	0	1	1	5	8	4	5	*
3C	81	0	0	0	1	2	*	*	*	*	*	55	0	1	9	*	3	3	1	3	1	7
3C	82	0	0	2	6	8	9	*	*	*	*	395	0	0	3	6	9	6	5	3	5	*
3C	83	·	·	0	6	9	*	*	*	*	*	307	·	·	0	1	4	9	*	6	4	6
3C	85	0	1	1	3	7	8	9	*	*	*	1070	0	0	2	3	3	7	*	*	7	7
3C	86	·	·	0	1	8	9	*	*	*	*	138	·	·	0	*	7	5	3	9	9	8
3C	88	·	0	0	1	8	9	*	*	*	*	381	·	0	2	5	2	3	7	6	8	*
5A	77	·	·	·	·	0	7	5	*	*	*	68	·	·	·	·	1	0	1	3	3	*
5A	78	·	·	·	·	0	·	*	*	*	*	43	·	·	·	·	0	·	0	0	0	*
5A	81	·	·	1	2	4	8	*	0	·	·	89	·	·	2	*	*	9	1	0	·	·
5A	83	·	·	0	5	7	*	*	*	*	*	184	·	·	0	5	8	*	7	4	2	6
5A	85	·	3	2	2	6	9	*	*	*	*	311	·	0	2	4	0	1	2	5	6	*
5A	88	·	·	1	7	*	*	*	*	*	*	113	·	·	8	3	1	2	7	*	6	8
5B	77	·	0	0	·	2	4	9	*	*	*	254	·	0	0	·	0	0	2	4	3	*
5B	78	·	·	0	0	3	*	*	*	*	*	46	·	·	0	2	1	0	1	1	1	*
5B	79	·	0	·	8	8	9	*	*	*	*	268	·	0	·	0	0	1	0	0	0	*
5B	80	·	·	0	0	0	*	9	*	*	*	250	·	·	7	*	1	4	9	7	4	7
5B	81	·	0	0	1	6	8	*	*	·	·	145	·	0	4	*	6	1	0	0	·	·
5B	82	0	0	1	1	8	*	*	*	*	*	425	0	0	0	1	3	8	7	2	1	*
5B	83	·	·	·	1	4	8	*	*	*	*	420	·	·	·	1	2	6	*	9	3	4
5B	85	·	0	0	1	3	9	9	*	*	*	586	·	0	5	4	1	2	6	7	7	*
5B	88	0	0	0	2	6	*	*	*	*	*	189	0	0	3	1	5	6	9	6	6	*

TABLE 4. Tabular histograms of percentage maturity at length (cm), by sex (M or F) and area. Sample sizes (N) and corresponding sample frequency histograms are also shown. The symbol for each histogram cell represents the cell value (percentage mature or sample frequency) on a scale of 0 to 10, where '*' indicates '10' and '·' indicates an absence of data. Data are grouped into 5-cm length intervals. The first interval includes data for lengths <50 cm, and the last interval includes data for lengths ≥90 cm.

		Percent mature at length										Sample frequency									
Sex	Area	50		60		70		80		90+	N	50		60		70		80		90+	
F	3C	0	1	1	3	8	9	*	*	*	*	2749	0	0	2	5	5	7	*	*	9	*
F	5A	·	3	1	3	5	9	*	*	*	*	808	·	0	2	4	4	4	4	5	4	*
F	5B	0	0	0	1	6	9	*	*	*	*	2583	0	0	2	3	2	4	6	5	3	*
M	3C	0	2	4	8	*	*	*	*	*	*	1706	0	0	5	7	9	*	7	3	1	0
M	5A	·	0	2	6	9	*	*	*	*	*	491	·	0	5	7	*	8	4	2	0	0
M	5B	0	0	1	5	9	*	*	*	*	*	1021	0	0	7	8	8	*	6	5	3	1

ther illustrate the conclusions from Table 4 that size at 20% maturity tends to be sex and area specific. A greater width to some of the profiles indicates greater uncertainty, and can be accounted for by low sample frequencies over the appropriate size range. In particular, there are insufficient data to determine the lower bound to the 95% confidence interval for male lingcod from Area 3C-N, that is $p < 0.95$ for $u_1 = 0$. Uncertainty in size at 20% maturity, relative to size at 80% maturity, is also evident from the 95% joint confidence regions in Fig. 5. Thus, three of the regions appear elongated along the u_1 axis. Most regions are nonoverlapping, indicating significant differences in size at maturity at the 95% probability level.

As noted in Table 2, fewer samples with length and age data are available than samples with length alone. This reduced data set must be used for multivariate analyses. To facilitate comparisons between univariate and multivariate analyses, we present parameter estimates in Table 8 for the univariate length model obtained from the reduced length–age data set, with data grouped by 2-cm length intervals. We use 2-cm rather than 1-cm groupings in multivariate analyses due to the larger number of possible cells. As for Table 7, we cannot reject the hypothesis that $b = 1$ against the alternative of an unrestricted

TABLE 5. Tabular histograms of percentage maturity at age, by sex (M or F) and area. Sample sizes (*N*) and corresponding sample frequency histograms are also shown. The symbol for each histogram cell represents the cell value (percentage mature or sample frequency) on a scale of 0 to 10, where '*' indicates '10' and '·' indicates an absence of data. Data for ages ≥10 are grouped in the last interval.

		Percent mature at age											Sample frequency									
Sex	Area	1	2	3	4	5	6	7	8	9	10+	*N*	1	2	3	4	5	6	7	8	9	10+
F	3C	0	0	3	7	9	*	*	*	*	*	942	0	1	4	7	8	*	7	4	2	2
F	5A	·	·	0	5	8	9	*	*	*	*	106	·	·	0	4	3	*	7	4	2	*
F	5B	·	0	1	6	8	9	*	*	*	*	1213	·	0	2	4	8	*	7	5	4	*
M	3C	·	6	4	8	9	*	*	*	*	*	539	·	0	4	6	*	*	9	5	3	3
M	5A	·	·	0	8	9	*	*	*	*	·	29	·	·	5	8	*	8	4	1	1	·
M	5B	·	0	0	2	7	9	9	*	*	*	571	·	0	4	6	*	*	9	4	3	4

TABLE 6. Tabular histograms of percentage maturity at length (cm), by sex (M or F), area, and age. Sample sizes (*N*) and corresponding sample frequency histograms are also shown. The symbol for each histogram cell represents the cell value (percent mature or sample frequency) on a scale of 0 to 10, where '*' indicates '10' and '·' indicates an absence of data. Data for each age are grouped into 5-cm length intervals. The first interval includes data for lengths <50 cm, and the last interval includes data for lengths ≥90 cm. Data for ages ≥8 are grouped in the last row for each sex and area combination.

			Percent mature at length										Frequency at length									
Sex	Area	Age	50		60		70		80		90+	*N*	50		60		70		80		90+	
F	3C	1	0	·	·	·	·	·	·	·	·	2	*	·	·	·	·	·	·	·	·	
F	3C	2	0	0	0	0	·	·	·	·	·	23	*	8	8	4	·	·	·	·	·	
F	3C	3	0	0	1	2	6	*	*	·	·	92	0	1	7	*	3	2	0	·	·	
F	3C	4	·	·	3	4	8	9	*	*	·	135	·	·	1	8	*	8	2	0	·	
F	3C	5	·	·	0	4	8	9	*	*	*	167	·	·	0	2	5	*	9	8	1	0
F	3C	6	·	·	0	6	8	*	*	*	*	207	·	·	0	1	2	4	9	*	7	1
F	3C	7	·	·	·	·	*	9	*	*	*	144	·	·	·	·	0	1	4	*	*	5
F	3C	8+	·	·	·	·	·	*	*	*	*	172	·	·	·	·	·	0	0	1	4	*
F	5B	2	·	·	0	0	·	·	·	·	·	4	·	·	*	*	·	·	·	·	·	
F	5B	3	0	0	0	0	0	*	*	·	·	*	60	0	0	*	*	2	0	0	·	·	0
F	5B	4	·	0	1	1	4	*	8	*	*	·	101	·	0	5	8	5	*	*	2	0	·
F	5B	5	·	·	0	0	7	9	9	*	*	*	194	·	·	1	3	1	5	*	7	4	1
F	5B	6	·	·	0	0	3	9	*	*	*	*	223	·	·	0	1	1	3	7	*	6	7
F	5B	7	·	·	·	0	*	9	*	*	*	*	170	·	·	·	0	0	2	3	4	6	*
F	5B	8+	·	·	·	0	·	*	*	*	*	*	461	·	·	·	0	·	0	0	0	0	*
M	3C	2	·	5	*	0	·	·	·	·	·	·	5	·	*	*	5	·	·	·	·	·	·
M	3C	3	·	2	3	6	·	·	·	·	·	·	40	·	1	*	6	·	·	·	·	·	·
M	3C	4	·	·	6	9	9	*	·	*	·	·	64	·	·	4	*	5	1	·	0	·	·
M	3C	5	·	·	7	9	9	*	*	·	·	·	108	·	·	0	7	*	7	2	·	·	·
M	3C	6	·	·	*	9	*	*	*	*	*	·	106	·	·	0	4	*	*	*	1	0	·
M	3C	7	·	·	·	8	*	*	*	*	*	·	96	·	·	·	1	2	*	*	4	0	·
M	3C	8+	·	·	·	·	*	*	*	*	*	*	120	·	·	·	·	0	1	9	*	3	0
M	5B	2	·	·	0	·	·	·	·	·	·	·	1	·	·	*	·	·	·	·	·	·	·
M	5B	3	·	0	1	0	·	·	·	·	·	·	46	·	0	*	2	·	·	·	·	·	·
M	5B	4	0	0	0	3	7	*	·	·	·	·	69	0	1	*	7	2	0	·	·	0	0
M	5B	5	·	·	1	4	9	9	*	*	·	·	115	·	·	4	6	8	*	0	0	·	·
M	5B	6	·	·	4	6	*	*	*	*	*	·	109	·	·	1	1	7	*	5	1	0	·
M	5B	7	·	0	0	8	8	9	*	*	*	*	98	·	0	0	1	4	5	*	6	1	0
M	5B	8+	·	·	·	·	5	*	*	*	*	*	133	·	·	·	·	0	1	4	*	8	1

b. With $b = 1$, estimates of size at 20%, 50%, and 80% maturity differ by at most 2 cm between comparable cases in Tables 7 and 8.

Analyses for the age–maturity relationship may proceed in a similar fashion to those above for the length–maturity relationship. Parameter estimates obtained from (1.10) for the age data are listed in Table 9. As discussed in conjunction with Table 5, age at 20% maturity for male lingcod is either younger (Area 3C-N) or older (Area 5B) than for female lingcod from the same area. For both sexes, age at 20% maturity is younger for fish from Area 3C-N than for fish from Area 5B. The age analysis differs from the length analysis, however, in that we can reject the hypothesis of $b = 1$ with 95% confidence for female lingcod from Area 5B. This shows that the canonical logit link function is not always appropriate.

Application of the multivariate model requires a different set of prescribed quantities. Here, we assign the subscripts 1 and 2 to quantities associated with length and age, respectively. We

TABLE 7. Parameter estimates for the univariate length–maturity model, including lengths (cm) at 20%, 50%, and 80% maturity, and minimum values of the likelihood function $l(\boldsymbol{\theta})$, by sex (M and F) and area. Values of the fixed parameters (α,γ_1,v,V) are (0,1,0.2,0.8). Cases are shown for each sex and area combination corresponding to fixed $b=1$ and b free. The case adopted with 95% confidence during hypothesis testing is indicated by '*'.

				Length at percent maturity			
Sex	Area	b	c_1	20%	50%	80%	$l(\boldsymbol{\theta})$
F	3C	1	−0.28	60.0	64.1	68.6	997.33*
F	3C	0.51	0.96	60.0	64.0	68.7	996.58
F	5A	1	2.79	59.9	65.5	70.3	358.47*
F	5A	2.21	0.38	59.9	65.6	70.2	358.07
F	5B	1	−1.86	63.4	66.5	70.1	740.98*
F	5B	0.67	−0.81	63.3	66.4	70.1	740.60
M	3C	1	5.49	51.3	58.1	62.5	712.13*
M	3C	3.30	1.69	51.1	58.1	62.5	711.79
M	5A	1	2.13	57.0	60.8	64.4	277.13*
M	5A	2.07	−0.56	56.9	60.9	64.3	276.97
M	5B	1	0.10	58.7	62.2	65.8	525.46*
M	5B	0.34	2.44	58.7	62.1	65.9	525.07

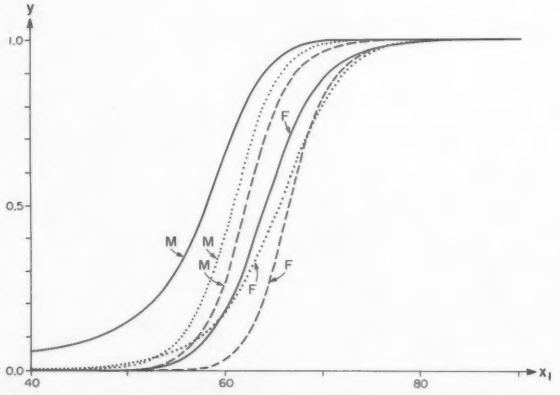

FIG. 2. Predicted relationships between maturity (y) and length (x_1) for male (M) and female (F) lingcod from Area 3C-N (solid line), 5A (dotted line) and 5B (dashed line). Curves are obtained from parameter estimates in Table 7 for cases with $b=1$.

choose $u_1=60$ cm, $U_1=70$ cm, $u_2=3$ yr, $U_2=6$ yr, $v=0.1$, and $V=0.9$, so that the three-dimensional rectangular region

$$(u_1,U_1) \times (u_2,U_2) \times (v,V),$$

includes most observed points (x_1,x_2,y). Based on our convention for x_1 and x_2, H_C represents the hypothesis associated with the univariate length model, and H_D the hypothesis associated with the univariate age model. For simplicity, we begin with the restriction $b=1$, as we could not distinguish $b=1$ from an unrestricted b in most univariate analyses.

Parameter estimates obtained from (1.10) for each of the five hypotheses outlined in section 1 are presented in Table 10. When $\gamma_1=0$ or $\gamma_2=0$, the problem is reduced to a univariate model, and the estimates for (c_1,c_2) and $l(\boldsymbol{\theta})$ are identical to the corresponding estimates in Tables 8 and 9. Note that both length and age together appear to be better predictors of maturation

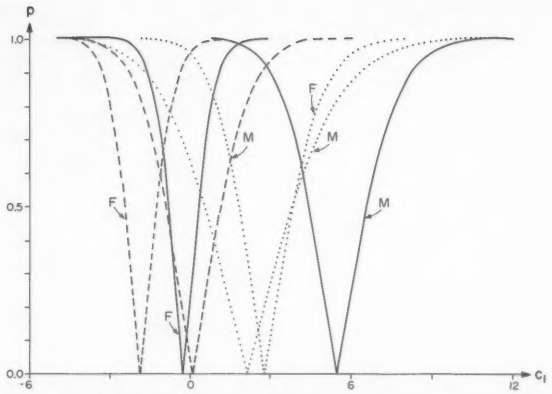

FIG. 3. Probability (p) profiles for the shape parameter c_1 in the univariate maturity–length model. Profiles (obtained from (1.13) with $b=1$) are shown for male (M) and female (F) lingcod from Area 3C-N (solid line), 5A (dotted line), and 5B (dashed line).

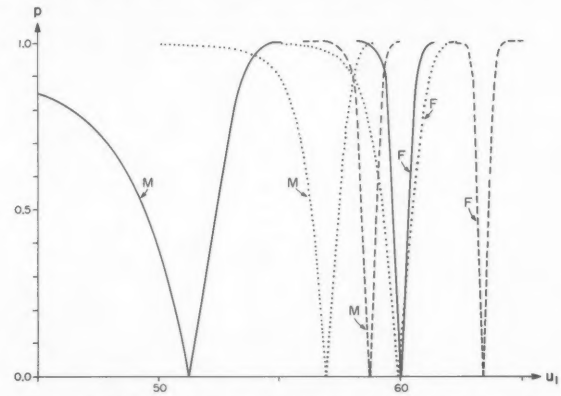

FIG. 4. Probability (p) profiles for length at 20% maturity (u_1) for male (M) and female (F) lingcod from Area 3C-N (solid line), 5A (dotted line), and 5B (dashed line). Profiles are obtained from (1.13) with $b=1$.

than either attribute alone. In all cases, it is possible to use (1.12) to reject both null hypotheses H_C and H_D in favor of the alternative hypothesis H_B. There are no cases for which the null hypothesis H_B can be rejected in favor of the alternative H_A. Furthermore, although not included in the table, the value of $l(\boldsymbol{\theta})$ with b unrestricted is similar in all cases to the corresponding value for hypothesis H_A. Hence, the choice $b=1$ in H_B cannot be rejected.

The topology of the case with $c_1=c_2$ is described in section 1 and illustrated in Fig. 6 for the four examples in Table 10. Tabular histograms are superimposed on each panel of the figure, corresponding to the data in Table 6, but using 10-cm rather than 5-cm length intervals. The five curves in each panel represent length–age contours for 10%, 30%, 50%, 70%, and 90% maturity. For three of the panels (A, B, and D), the curves are convex up and asymptotic to both axes, with $c<0$. For Fig. 6C, the curves are convex down, intersecting both axes with $c>1$. Differences in the value of c reflect differences in the underlying data. The data for Fig. 6C include age-2, 50-cm fish that are 50% mature; for other panels there are either no data in that region or fish of that age and size are all immature.

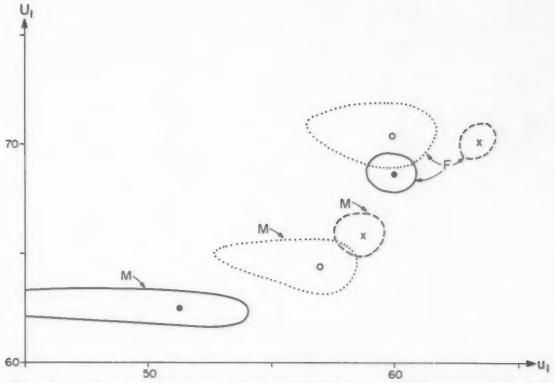

FIG. 5. Estimates and approximate 95% joint confidence regions for length (cm) at 20% (u_1) and 80% (U_1) maturity for male (M) and female (F) lingcod from Area 3C-N (solid line, closed circle), 5A (dotted line, open circle), and 5B (dashed line, cross). Regions are obtained from (1.12) with $b=1$.

TABLE 8. Parameter estimates for the univariate length–maturity model, including lengths (cm) at 20%, 50%, and 80% maturity, and minimum values of the likelihood function $l(\boldsymbol{\theta})$ by sex (M and F), and area. The data differ from those used for Table 7 in that the reduced length–age data set is used. Values of the fixed parameters (α,γ_1,ν,V) are (0,1,0.2,0.8). Cases are shown for each sex and area combination corresponding to fixed $b=1$ and b free. The case adopted with 95% confidence during hypothesis testing is indicated by '*'.

				Length at percent maturity			
Sex	Area	b	c_1	20%	50%	80%	$l(\boldsymbol{\theta})$
F	3C	1	0.65	59.9	63.6	67.4	306.98*
F	3C	1.20	0.13	59.9	63.6	67.4	306.96
F	5B	1	−1.50	64.6	67.5	70.7	206.52*
F	5B	0.73	−0.54	64.6	67.4	70.8	206.42
M	3C	1	4.93	49.7	57.1	62.0	185.61*
M	3C	0.12	6.64	50.2	57.1	62.0	185.59
M	5B	1	−2.17	60.2	62.8	65.8	217.50*
M	5B	1.11	−2.58	60.2	62.8	65.8	217.49

Sex and area differences can be examined by comparing the contours among panels in Fig. 6. Consider the middle contours, for example. These represent curves along which size at 50% maturity depends on age. The 50% maturity contour for female lingcod from Area 3C-N is shifted to smaller sizes relative to the contour for female lingcod from Area 5B, and to larger sizes relative to the contour for male lingcod from Area 3C-N.

The relative contribution of length and age to the multivariate model can be examined from approximate 95% joint confidence regions for γ_1 and γ_2 in Fig. 7. All of the regions overlap to some extent near the line $\gamma_2=0.2$, and the region for female lingcod from Area 5B includes a segment of the line $\gamma_2=0$ near the lower bound. In contrast, none of the regions are located near $\gamma_1=0$. Hence, length contributes relatively more information than age to the multivariate model. In addition, γ_1 appears to be better determined than γ_2 for three of the regions. For the fourth region, corresponding to male lingcod from Area 3C-N, γ_1 appears to be less well determined, reflecting the uncertainty evident in Fig. 5 in the length at which maturation begins. The fourth region also includes the lowest values of γ_1. This is the case for which there is the least difference in $l(\boldsymbol{\theta})$ between values related to H_C and H_D (Table 10).

TABLE 9. Parameter estimates for the univariate maturity–age model, including age at 20%, 50%, and 80% maturity, and minimum values of the likelihood function $l(\boldsymbol{\theta})$, for lingcod maturity–age relationships by sex (M and F), and area. Values of the fixed parameters (α,γ_1,ν,V) are (0,1,0.2,0.8). Cases are shown for each sex and area combination corresponding to fixed $b=1$ and b free. The case adopted with 95% confidence during hypothesis testing is indicated by '*'.

				Age at percent maturity			
Sex	Area	b	c_1	20%	50%	80%	$l(\boldsymbol{\theta})$
F	3C	1	−0.12	3.3	3.9	4.8	444.43*
F	3C	−2.40	2.73	3.4	3.8	4.8	441.54
F	5B	1	−0.28	3.8	4.5	5.4	536.44
F	5B	−1.70	2.54	3.6	4.3	5.5	531.05*
M	3C	1	1.04	2.4	3.5	4.5	200.48*
M	3C	2.99	0.05	2.4	3.5	4.5	199.93
M	5B	1	−0.82	4.4	5.0	5.9	349.26*
M	5B	1.85	−1.67	4.4	5.0	5.9	348.78

TABLE 10. Parameter estimates for the multivariate length–age maturity model, and minimum values of the likelihood function $l(\boldsymbol{\theta})$, for lingcod maturity relationships by sex (M and F) and area. Values of the prescribed quantities $(b,u_1,u_2,U_1,U_2,\nu,V)$ are (1, 60, 3, 70, 6, 0.1, 0.9). Four cases are shown for each sex and area combination corresponding to four possible hypotheses. The case adopted with 95% confidence during hypothesis testing is indicated by '*'.

Sex	Area	H	c_1	c_2	α	γ_1	γ_2	$l(\boldsymbol{\theta})$
F	3C	H_C	—	−0.12	0.030		1.159	444.43
F	3C	H_D	0.65	—	0.193	0.837	0	306.98
F	3C	H_B	−0.17	−0.17	0.044	0.749	0.321	300.00*
F	3C	H_A	1.41	−4.71	−0.135	0.726	0.480	298.31
F	5B	H_C	—	−0.28	−0.243	0	1.234	536.44
F	5B	H_D	−1.50	—	−0.405	1.152	0	206.52
F	5B	H_B	−1.94	−1.94	−0.768	1.111	0.472	201.68*
F	5B	H_A	−1.41	−4.77	−1.056	1.072	0.785	201.33
M	3C	H_C	—	1.04	0.351	0	0.921	200.48
M	3C	H_D	4.93	—	0.672	0.916	0	185.61
M	3C	H_B	1.94	1.94	0.534	0.559	0.361	179.25*
M	3C	H_A	7.59	0.84	0.466	0.697	0.413	177.64
M	5B	H_C	—	−0.82	−0.855	0	1.707	349.26
M	5B	H_D	−2.17	—	0.165	1.012	0	217.50
M	5B	H_B	−1.89	−1.89	−0.364	0.863	0.665	209.76*
M	5B	H_A	−1.67	−2.06	−0.388	0.865	0.689	209.74

An earlier examination of size at maturity for lingcod off British Columbia was conducted by Wilby (1937). He found male and female lingcod to be fully mature at lengths of 45 and 67 cm, respectively. His estimate for male lingcod is considerably smaller than those in Tables 7 and 8. However, he does not describe his maturity data, and we canot interpret the differences between his estimates and ours.

A better comparison is provided by Miller and Geibel (1973), who present maturity–length data for lingcod collected off central California. If we apply our univariate length–maturity model to their data, we obtain the estimates given in Table 11. Since British Columbia is located to the north of California, the estimates are consistent with our observation of an increasing size

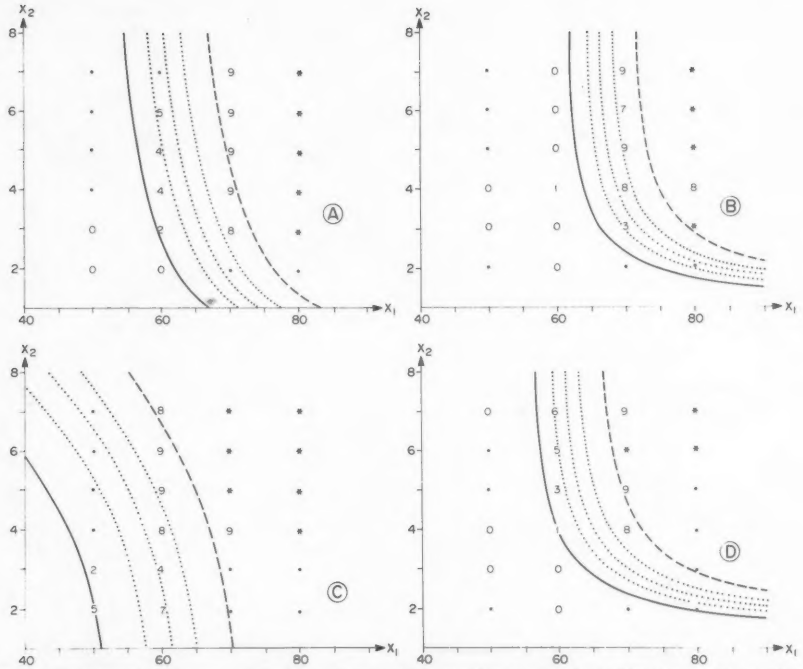

FIG. 6. Predicted length–age contours for 10% (solid line), 30% (dotted line), 50% (dotted line), 70% (dotted line), and 90% (dashed line) maturity for (A) female lingcod from Area 3C-N, (B) female lingcod from Area 5B, (C) male lingcod from Area 3C-N, and (D) male lingcod from Area 5B. Contours are obtained from parameter estimates corresponding to H_B in Table 10. The superimposed characters represent tablular histograms of percentage maturity at length and age (as in Table 6), with data for each age grouped into 10-cm length intervals.

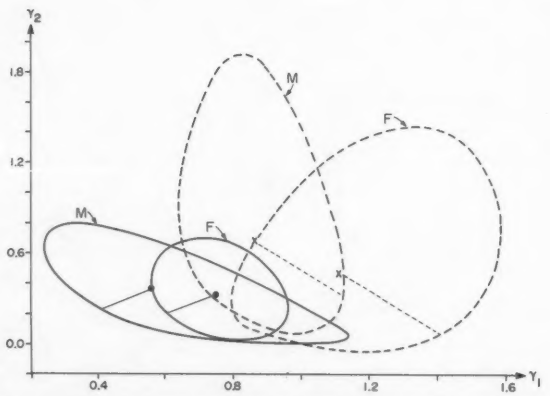

FIG. 7. Estimates and approximate 95% joint (γ_1,γ_2) confidence regions for male (M) and female (F) lingcod from Area 3C-N (solid line, circle) and 5B (dashed line, cross). For clarity, thin lines connect point estimates to region boundaries. Regions are obtained from (1.12) with parameter estimates corresponding to H_B in Table 10.

at maturity with increasing latitude. Similar latitudinal trends in size and age at maturity have been shown for a number of fish species (Gunderson 1977; Beacham 1982, 1983; Haldorson and Love 1990).

Note that for male lingcod from California, it is possible to reject the hypothesis of $b=1$ with 95% confidence in favor of an unrestricted b. In addition, both exponents b and c_1 are large in magnitude. Exponents of this magnitude are generally indicative of a discontinuity in the sample data. In this case, all fish of a length less than and including 38 cm are immature, and the three fish sampled with a length of 39 cm are all mature. The predicted curve becomes very steep between these lengths, jumping between 0% mature at 38 cm and 46% mature at 39 cm.

Miller and Geibel (1973) also report age at 50% maturity to be near 2 yr for male lingcod from California and near 5 yr for females. The data for males are again consistent with a latitu-

TABLE 11. Parameter estimates from the univariate length–maturity model for the data of Miller and Geibel (1973) for male (M) and female (F) lingcod from central California. Estimated lengths (cm) are given at 20%, 50%, and 80% maturity, along with minimum values of the likelihood function $l(\boldsymbol{\theta})$. Values of the fixed parameters (α,γ_1,ν,V) are (0,1,0.2,0.8). Cases are shown for each sex corresponding to fixed $b=1$ and b free. The case adopted with 95% confidence during hypothesis testing is indicated by '*'.

			Length at percent maturity			
Sex	b	c_1	20%	50%	80%	$l(\boldsymbol{\theta})$
F	1	−1.48	53.2	58.8	66.2	100.57*
F	−2.20	7.10	51.7	59.0	68.8	97.35
M	1	−0.73	36.9	42.4	49.5	94.29
M	−10.80	17.36	38.0	39.8	51.8	88.82*

dinal trend in increasing age at maturity. This is not true for the female data, however. The older age at maturity for female lingcod obtained by Miller and Geibel (1973) could be explained by sample bias. As noted in section 1, age at maturity obtained from samples could be biased if the fishery from which the samples were obtained is size-selective. This is almost certainly true for lingcod fisheries. Alternatively, differences could reflect slower growth of California lingcod, or a difference in ageing techniques.

In summary, it appears that the multivariate model (1.1)–(1.4) provides a flexible tool for comparing a variety of maturity relationships. Here, for example, we find both length and age to be useful maturity attributes, whose joint contribution to the model gives significantly better predictions of percentage maturation than either attribute alone. The model need not be limited to maturity applications, however. It could be applied in any context where a probabilistic response y in the range $(0,1)$ is monotonically related to positive attributes x_i $(i = 1,..,k)$. For example, y could represent a survival probability in a toxicity study, where survival steadily increases or decreases with an increase in x_i. This paper, together with earlier works by Schnute and Jensen (1986) and Schnute and Richards (1990) offers an integrated approach to univariate or multivariate models of maturity and survivorship.

Acknowledgements

Motivation to undertake this work came partly from our colleague Alan Cass, who drew our attention to variable lingcod maturity patterns. We are grateful to him and to various port samplers for collecting the sample data described here. Comments by two anonymous reviewers have improved our presentation.

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Growth of 0+ Roach (*Rutilus rutilus*) in Relation to Temperature and Size in a Shallow Eutrophic Lake: Comparison of Field and Laboratory Observations

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The growth rates of larval and juvenile roach (*Rutilus rutilus*) were measured in the laboratory at different temperatures under conditions of excessive food supply. Using these data, the maximum growth rate of 0+ roach in relation to size and water temperature could be adequately described with an equation of the type: $dW/dt = a_{max} \cdot W^b \cdot (T - c)$ (W = weight, T = temperature, t = time). Using this equation the growth of larval and juvenile roach in Tjeukemeer was predicted for 12 successive years. The predicted growth rates were about 20% lower than the observed growth rates. Reasons for this bias are discussed. The results indicate that food is not limiting growth of roach in Tjeukemeer.

Les taux de croissance des gardons (*Rutilus rutilus*) juvéniles et larvaires ont été mesurés en laboratoire à diverses températures sous des conditions d'alimentation excessive. À partir de ces données, le taux de croissance maximum des gardons d'âge 0+ en fonction de la taille et de la température de l'eau pourrait être décrit de façon adéquate à l'aide d'une équation du type : $dW/dt = a_{max} \cdot W^b \cdot (T - c)$ (où W = poids, T = température, t = temps). À l'aide de cette équation, la croissance des gardons juvéniles et larvaires a été prédite à Tjeukemeer pour 12 années successives. On a montré que les taux de croissance prévus étaient d'environ 20 % plus faibles que les taux observés. Les auteurs traitent des raisons de cette différence. Les résultats indiquent que la nourriture ne constitue pas un facteur de limitation de la croissance des gardons à Tjeukemeer.

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A central question in fish ecology is whether recruitment of a species may be controlled by food limitation, by predation, or by a combination of these factors (e.g. May 1974; Cushing 1981; Elliott 1984, 1987; Rothschild 1986; McNamara and Houston 1987). If food is limited, there should be a relationship between growth and prevailing food conditions. However, growth is also known to be affected by water temperature and size of the animal (Elliott 1975a; Brett 1979; Wieser et al. 1988) and, therefore, the effect of these factors should be analyzed simultaneously.

Usually, growth data are analyzed in a multiple regression, with size as the dependent variable and fish abundance, mean water temperature, or the number of day degrees, as independent variables (e.g. Broughton and Jones 1978; Rauck and Zijlstra 1978; Henderson 1985; Henderson and Brown 1985; Muller and Meng 1986). If there is a significant relation between water temperature and size, and no relation between abundance of fishes and size, it is generally concluded that food is not a factor limiting recruitment. This approach has several disadvantages. Food, environmental temperature, and size of the animal vary seasonally, and size at the end of the season will result from continuous interactions among these factors, and not (as is implicitly assumed in the regression analysis) of interactions between mean values over the observed period. Moreover, as the effect of size on the growth rate is not incorporated into the regression model, only growth of animals of the same size can be analyzed. As the functional relationship between growth rate, food conditions, temperature, and size remains unclear, prediction of the size of an animal at any moment under a given set of environmental conditions is not possible.

Many authors have tried to model growth of fishes (reviews by Brett 1979; Ricker 1979; Ursin 1979). Some of these models must be considered descriptive. In general, they fit a sigmoid curve, e.g. the logistic curve, either to the observed growth during a life span (Bayley 1977; Bagenal and Braum 1978) or to growth observed during one season (Lockwood 1974; Guma'a 1978; Tong 1984). Functional relationships between growth rate and its controlling variables are not incorporated into these models. Several attempts have been made to model growth based on physiological processes (von Bertalanffy 1957; Ursin 1979; Jobling 1985). These models currently have limited application to natural systems. This situation prompted us to seek a compromise between the descriptive and the functional approaches. Absolute instanteous growth rate (dW/dt) is considered as the multiplicative effect of functions of the three independent variables: food, weight, and temperature (cf. Brett 1979).

We developed a dynamic model for the growth rate of 0+ roach, based on relations among growth rate, temperature, and size under excess food conditions. Parameters were estimated

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by rearing fish in the laboratory under controlled temperature conditions with excessive feeding (cf. Elliott 1975a; Jobling 1980; Wieser et al. 1988). If water temperatures in the lake are known, the model can be used to predict size of the fish. A comparison of expected size with observed size should answer whether 0+ roach in Tjeukemeer are food limited. Earlier, Zijlstra et al. (1982) used a similar approach for plaice (*Pleuronectes platessa*), based on the experimental work of Fonds (1979).

Materials and Methods

Rearing Roach at Maximum Rations

Adult specimens of roach were obtained from eutrophic waters in the northwest of the Netherlands and stripped. After fertilization eggs were kept in a funnel. Water was refreshed continuously. After development of the eye pigment, eggs were placed at the bottom of glass aquaria (40×40×35 cm) with a continuous flow of water. From the onset of exogenous feeding larvae were fed in excess with *Artemia* nauplii by adding nauplii automatically to the ciruclating water at 10-min intervals (Wieser et al. 1988). Larvae were kept at ≈15°C prior to the 33rd day after hatching. Thereafter, larvae were reared at four temperatures: approximately 13, 17, 20, and 24°C (see Appendix). The transition to the new temperatures occurred over 4 h. Larvae were then fed in excess on live zooplankton, collected from Tjeukemeer with hoopnets (Tong 1984). Initially, we used a net with a 250-μm mesh which ensured that the food consisted mainly of the smaller cladoceran species *Bosmina longirostris, B. coregoni, Chydorus sphaericus*, and copepodite stages of cyclopoid copepods. Large zooplankton were excluded by towing a 500-μm net in front of the 250-μm net. As the fish grew, we gradually switched to larger food consisting mostly of *Daphnia hyalina* and adult cyclopoid copepods until the 67th day after hatching. After day 67, we fed frozen zooplankton for 2 wk. Because growth sharply declined, live chironomid larvae were subsequently used as food, until the 178th day after hatching. Fish biomass in the aquaria never exceeded 1 $g \cdot L^{-1}$ fresh weight.

Temperatures in the aquaria were measured three to four times a week during the first 80 d and weekly thereafter. Weights and lengths of the fish in each aquarium were measured 11 times. Between four and 27 fishes were collected using a small net and fork length of each specimen was measured to the nearest millimetre. Adhering water was removed before measuring the fresh weight to three significant digits (see Appendix).

The linear regression relationships between the log-transformed means of lengths and weights were calculated. These relationships are assumed to change at the end of morphogenesis, at ≈19 mm (see also Tong 1984 and Rheinberger et al. 1986).

Definition of the Growth Model and Parameter Estimation

The absolute instantaneous growth rate can be viewed as the multiplicative effect of three independent functions f, g and h:

$$(1) \quad G = dW/dt = f(F) \cdot g(W) \cdot h(T),$$

where F represents the food conditions at time t, T the water temperature and W the weight. The weight increment in a certain period t can be calculated from the following integral:

$$(2) \quad W_t - W_0 = \int_0^t G\,dt = \int_0^t f(F) \cdot g(W) \cdot h(T)\,dt.$$

To avoid problems with solving this integral, the weight increment was approximated by a summation of weight increments over short periods of time (time-step in the model was 1 d), in which the values of $f(F)$, $g(W)$, and $h(T)$ were assumed to be constant. The reliability of this approximation will, of course, increase with decreasing length of Δt. Also, using the geometric mean weight during the period Δt, instead of the initial weight, improves the approximation. This improvement is achieved by an iterative estimation of the weight increment during Δt after a first estimation from the initial weight, until this increment remains constant.

$f(F)$ is assumed to be a constant for any given set of food conditions (Elliott 1975a; Brett 1979; Borgmann and Ralph 1985):

$$(3) \quad f(F) = a.$$

At excess food, parameter a should have its maxium value (a_{max}). The relation between growth and weight was assumed to be:

$$(4) \quad g(W) = W^b.$$

At temperatures above a lower threshold, but below the optimum temperature, a linear relationship between temperature and growth is assumed:

$$(5) \quad h(T) = T - c.$$

If $T<c$ weight is assumed to be constant, c representing the lower threshold temperature for growth. Combining equations (2), (3), (4), and (5), the expected final weight ($W_{t,exp}$) can be calculated, given an initial weight (W_0) and temperature data, using:

$$(6) \quad W_{t,\exp} = W_0 + \sum_{t\in(1/2\Delta t,\ 1\frac{1}{2}\Delta t,\ \ldots,\ t-1/2\Delta t)} a_{\max} \cdot W_t^b \cdot (T_t - c)$$

Elliott (1975a) used this relationship in his study on growth of brown trout (*Salmo trutta*). Instead of solving this differential equation (1) analytically, like Elliott, we solved it numerically. The optimal fit of the parameters a, b, and c for n observations was defined as the values of a, b, and c for which the sum of squares of the differences between the natural logarithms of the observed and expected weights

$$(7) \quad \sum_1^n (\ln(W_{t,\exp}) - \ln(W_{t,obs}))^2,$$

was minimal; a, b, and c were approximated with an iterative optimalisation algorithm using 0-GROWTH, a programme for a personal computer (available from the first author).

Collection of the Growth Data from Tjeukemeer

Mean lengths of 0+ roach in Tjeukemeer were obtained from fish caught in the open water zone using a 5-m beam trawl with a mesh size in the cod-end of 5.5 mm knot to knot. For each year in the period 1976–87, size data collected around 1 August and during the second week of October were used. For 1978, for which an extensive data set was available, 14 observations in the period 5 July–26 October were analyzed separately.

Estimating Water Temperature from Air Temperature

Because records of water temperature were incomplete, a second model was developed to predict water temperatures from

air temperatures. A simple relationship was assumed for a shallow, wind exposed, and holomictic lake.

The change in water temperature was assumed to result from the additive effects of conduction and radiation (Jeppesen and Iversen 1987). A linear function was fitted to describe the change in water temperature resulting from the difference between water and air temperature. As no radiation data were available, a mean daily value was approximated by a sinus with a period of 1 yr:

$$(8a)\quad T_{w,t} - T_{w,(t-\Delta t)} = e\cdot(T_{a,t} - T_{w,(t-\Delta t)}) +$$

$$(8b)\quad f + g\cdot\sin(2\pi\cdot(\text{daynr} - h)/365.25),$$

in which $T_{w,t}$ is the water temperature at time t, $T_{a,t}$ the air temperature at time t, daynr the number of the day as counted from the first day of January onwards and e, f, g, and h are constants. The first part of this formula (8a) represents the change in temperature caused by conduction, while the second part (8b) represents the influence of radiation. Parameters were estimated using a method similar to that used for the growth model. Best fit was defined as the minimal value of the sum of squares of the differences between the available water temperatures and the corresponding estimated values using an iterative algorithm (also available from the first author).

The total data set for water temperatures during 1971–87, used for optimalisation, consists of 587 water temperature measurements (de Boer et al. 1988). Air temperatures were obtained from a nearby station of the Royal Dutch Institute of Meteorology. The interval between individual measurements was 3 h.

Calculation of the Predicted Weight of 0+ Roach

If initial weight and time of onset of growth are known, the seasonal change of weight can be calculated with equation (6). Initial weights of fish in Tjeukemeer and in the laboratory experiment were assumed to be equal; the duration of egg and yolk sac stages in days (n) was predicted with the day–degree formula (e.g. Herzig and Winkler 1986; Elliott et al. 1987):

$$(9)\quad \sum_{t=1}^{n} (T - c) = d,$$

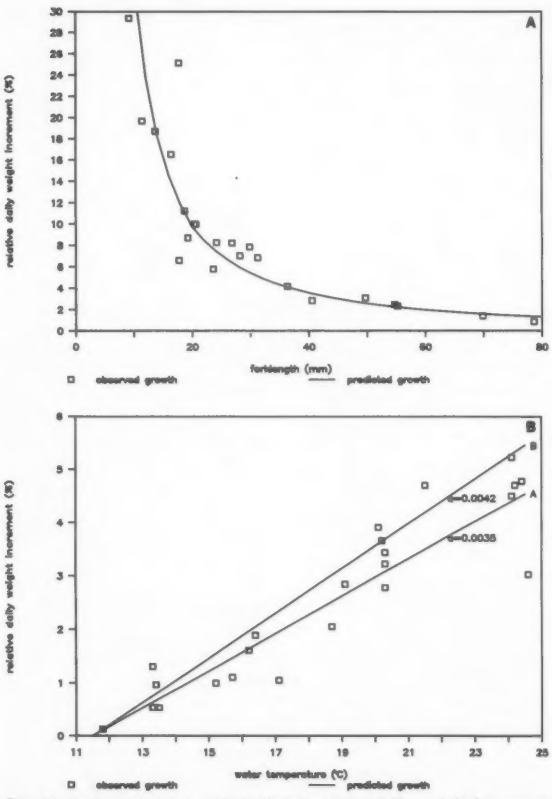
in which T represents environmental temperature at time t, c is the minimal temperature of development, d the total heat–sum needed for the onset of growth and n is (in days) the summed duration of the egg- and yolk-sac stages. For parameter c, the same value was arbitrarily assumed as for c in equation (6) obtained from the aquarium experiment, while parameter d was estimated from the duration of the egg- and yolk-sac stage, again in the laboratory experiment.

Calculation of the Food Parameter a

Values of a, b, and c were estimated from growth experiments. However, food conditions in the field will undoubtedly be different from the laboratory and therefore it is useful to estimate the food parameter a additionally on the basis of successive field data on size, assuming that b and c remain unchanged.

$$(10)\quad a = \frac{dW/dt}{W^b\cdot(T - c)}$$

The resulting value, called 'weight- and temperature-corrected growth rate', is used to compare growth rates of fish populations under different environmental conditions. Given the linear relationship between growth and temperature and a time-step of 1 d, parameter a represents the daily weight increment in grams for a fish of 1 g at a water temperature of 1°C above the minimum temperature.



The Netherlands. FIG. 1. (A) Predicted relative daily weight increment (——) in relation to size at 20°C. Observed relative growth rates (□) of each observation (i) of fish in the laboratory under excessive feeding conditions are correlated for the influence of temperature: $(dW/dt)/Wi = (a_i\cdot W_i^{0.56}\cdot(20-11.5))/W_i$. (B) Predicted relative daily weight increment in relation to temperature at a weight of 1 g (—— A: a_{max} = 0.0035). The optimal linear growth–temperature relationship for Tjeukemeer (—— B: a_{max} = 0.0042) is also shown. Observed relative growth rates (□) of fish in the laboratory under excessive feeding conditions are corrected for the influence of weight: $(dW/dt)/W_i = (a_i\cdot(T_i - 11.5)$.

Results

Growth Experiment for Roach at Maximum Rations

The total data set consisted of 31 observations for fish in the length range of 8–87 mm, and the temperature range of 12–24°C (see Appendix). Twenty-three observations (four observations during change of water temperature and four observations on fish fed with frozen zooplankton were not taken into account, see Materials and Methods) were used to fit the food parameter a_{max}, the weight parameter b and the temperature parameter c (equation (6)). Parameters b and c were estimated

TABLE 1. Mean fork lengths (L, mm), number of measured fishes (n) and standard deviation (SD, mm) of roach in Tjeukemeer around 1 August and in October. na = data not available, *; i = interpolated, e = extrapolated.

August					October			
Date	L	SD	n	*	Date	L	SD	n
29/07/76	53.3	3.20	164					
27/07/77	32.0	2.56	411					
01/08/77	32.7			i				
03/08/77	33.0	3.30	125					
26/07/78	34.9	2.09	9					
01/08/78	37.8			i	26/10/78	52.3	3.66	55
02/08/78	38.7	3.10	163					
01/08/78	36.3	2.18	69		24/10/79	49.9	2.99	17
30/07/80	35.8	2.51	6					
01/08/80	37.5			i	08/10/80	55.3	3.32	133
06/08/80	41.6	3.33	182					
22/07/81	37.8	2.50	6					
01/08/81	40.3			i	28/10/81	58.2	3.50	50
05/08/81	41.3	2.60	36					
21/07/82	44.0	na	na					
01/08/82	50.3			i	27/10/82	63.0	na	na
04/08/82	52.0	na	na					
20/07/83	37.0	2.59	272					
01/08/83	46.4			i	29/09/83	63.0	6.30	36
03/08/83	48.0	3.36	58					
25/07/85	37.1	3.10	57					
01/08/85	40.2			i	23/10/85	51.8	6.15	14
22/08/85	49.5	3.73	120					
01/08/87	37.9			e				
05/08/87	39.2	3.31	13		14/10/87	54.4	5.27	8
08/09/87	50.6	4.60	22					

to be 0.56 and 11.5°C, respectively, while the constant maximum value of the food function a_{max} was found to be 3.54 $g^{0.44}\cdot°C^{-1}\cdot d^{-1}\cdot 10^{-3}$. The explained variance in the instantaneous growth rate is 91.2% ($p<0.001$).

Deviations of each of the observations from the value predicted by the model were analyzed to evaluate the fit of equation (6) to the data. To do so the value of the food parameter was calculated for each individual observation i following equation (10). The standard deviation among these values of parameter a was 1.1 $g^{0.44}\cdot°C^{-1}\cdot d^{-1}\cdot 10^{-3}$. The fit of the weight–growth relation is visualized by plotting the observed relative daily weight increments together with the predicted values against size (Fig. 1A). The performance of the temperature function is shown by plotting the observed relative daily weight increments together with the predicted values against temperature (Fig. 1B, line A).

The deviations in Fig. 1B suggest that there might be some curvilinear relationship between growth and temperature, with a more than proportional increase of growth rate at higher temperatures. Adding a second order term to the temperature–growth equation (5), however, did not result in a smaller residual sum of squares.

Observed Size of 0+ Roach in Tjeukemeer

The approximate sizes of roach in the open water zone of Tjeukemeer on August 1, during the period of 1976–87, are shown in Table 1. Except for 1976 and 1979, size at August 1st had to be estimated by interpolating or extrapolating the results of two samplings close to August 1st.

The end of the growing season was assumed to be the second week of October when water temperatures dropped below 10°C.

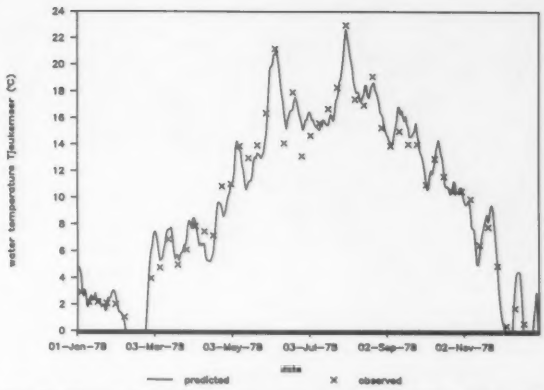


FIG. 2. Observed and predicted water temperatures (°C), based on air temperatures, in the open water zone of Tjeukemeer in 1978.

As there were no data from October 1983, the last observation in September had to be used.

Because catches were too small, no size data on roach were available from August 1984 and 1986, and from October 1976, 1977, 1984, and 1986.

For further calculations, fork lengths (mm) were converted to weights (in grams fresh weight), using regression relationships between mean lengths and mean fresh weights from the laboratory experiments. For fish <19 mm, this relation was $W=6.45\cdot10^{-7}\cdot L^{3.87}$, and for fish >19 mm $W=4.46\cdot10^{-6}\cdot L^{3.23}$.

Parameter Estimation for the Water Temperature Model

Best fit for the water temperature model was achieved with the formula:

$$T_{w,t} = T_{w,(t-3h)} + 0.034\cdot(T_{a,t} - T_{w,(t-3h)}) + 0.036 + 0.062\cdot\sin(2\pi\cdot(\text{daynr}-80.8)/365.25),$$

(for explanation of the variables see equation (8)). The model was free to choose the day with maximum radiation (parameter h, equation (8)): the value of the 20th of June corresponds closely to maximum day length.

The mean deviation of the model was 0.7°C, with 90% of the predicted water temperatures being within a range of 1.5°C from the observed temperatures. The maximal deviation was 3°C. The modeled temperatures for 1978 are shown as a line in Fig. 2, together with the observed water temperatures.

Expected and Observed Weights of 0-group Roach in Tjeukemeer

Expected weights were calculated from the predicted water temperatures using equation (6), and the values of a_{max}, b, and c as estimated in our experiments.

The durations of the egg and yolk sac stages (equation (9)) were estimated from the laboratory experiment and for parameter d a value of 45.5 was found. An initial weight of 0.001 g was assumed, making no correction for the influence of temperature on initial weights during the egg and yolk sac stages (Bengtson et al. 1987). Because the model is relatively insensitive to changes in either parameter d or initial weight, we did not estimate these more precisely.

Following conversion of weights back to lengths, expected lengths were compared with the observed lengths for the lab-

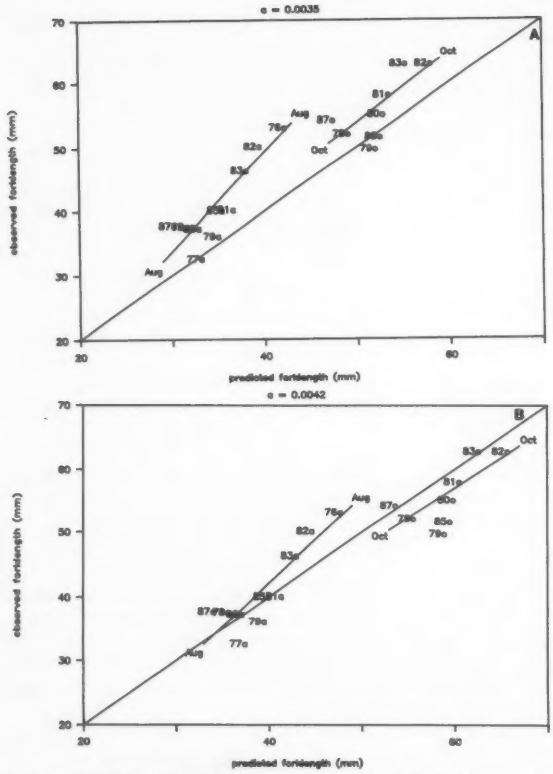


FIG. 3. (A) Predicted and observed sizes of roach in Tjeukemeer in the different years (a_{max} = 0.0035). Predictions for August (a) and October (o) were made from the beginning of the season on. For both data sets (August and October) functional regression lines between observed and predicted values are shown. (B) Same as in Fig. 3A, but now predictions were made with the optimal value of the food parameter for Tjeukemeer (a_{max} = 0.0042).

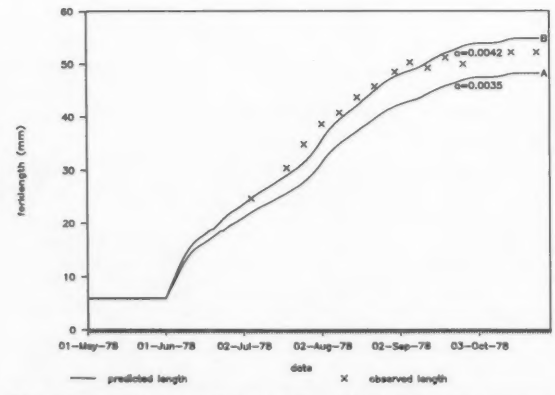


FIG. 4. Observed and predicted lengths of roach during the course of 1978. The predictions for both values of the food parameter a (line A: a_{max} = 0.0035, line B: a_{max} = 0.0042) are shown.

TABLE 2. Weight and temperature corrected growth rates ($g^{0.44} \cdot °C^{-1} \cdot d^{-1}$) of roach for different waterbodies and different periods. Weight- and temperature-data on Pitburger See, Seefelder See, and Stophenreuth after Wieser et al. 1988.

Waterbody	Period		a^* 1000
Aquarium	All observations		3.5
Tjeukemeer	All observations		4.2
Tjeukemeer	Onset growth–1 August		4.5
	Onset growth–October		3.9
Tjeukemeer	Onset growth–1 August	1976	5.1
		1977	3.5
		1978	4.8
		1979	3.8
		1980	4.4
		1981	4.1
		1982	5.1
		1983	4.9
		1985	4.4
		1987	5.2
Tjeukemeer	Onset growth–October	1978	3.9
		1979	3.5
		1980	3.8
		1981	4.1
		1982	4.0
		1983	4.3
		1985	3.5
		1987	4.4
Tjeukemeer	5 July – 19 July	1978	5.8
	19 July – 26 July	1978	7.8
	26 July – 2 August	1978	4.1
	2 August – 9 August	1978	2.4
	9 August – 16 August	1978	4.7
	16 August – 23 August	1978	3.1
	23 August – 31 August	1978	4.4
	31 August – 6 September	1978	7.0
	6 September – 26 October	1978	1.3
Pitburger See	25 May – 29 August	1984	2.5
Seefelder See	12 June – 5 September	1984	1.6
Stophenreuth	6 June – 5 September	1984	2.5
	31 May – 1 October	1985	2.1

TABLE 3. Number of roach, planktivorous 0+ fish, and piscivorous 0+ pikeperch per unit effort in August in Tjeukemeer. na = not available.

Year	Roach	0+ Planktivorous	0+ Piscivorous
1976	450	2285	34
1977	35	5446	0
1978	8	1014	0
1979	7	1117	0
1980	1777	7311	27
1981	62	17206	79
1982	651	2811	13
1983	1525	2703	na
1984	0	na	0
1985	30	1895	2
1986	na	na	na
1987	2	1347	4

oratory and field data sets (Fig. 3A). In both cases the onset of growth was taken as the starting point for the predictions.

A significant part of the variation, 50–75%, in size between years is explained by the model for both August 1st ($r=0.87$, $P<0.001$) and the end of the growing season ($r=0.72$, $P<0.05$). Functional regression lines for both data sets are shown in Fig. 3A. In all cases, however, growth in Tjeukemeer was underestimated by the model based on laboratory growth. The same holds for the predicted growth and observed growth in 1978 (Fig. 4). There is a very good relationship between the shapes of the observed and the expected growth curves, but an overall underestimation of size by the model.

Food Parameters from Field Data

For several sets of field data, values of the food parameter a, estimated by applying equation (10) are given in Table 2. In Tjeukemeer, the value of parameter a was on average $4.2 \cdot 10^{-3}$, which is 20% higher than in the aquarium experiments. Corresponding predicted lengths are shown in Fig. 3B and 4, line B. For the August data food parameter a was significantly higher then in the aquarium (t-test, $p<0.05$), but for the October observations there was no significant difference.

A possible relationship between growth rate of roach and its abundance, as a result of competition for food, was investigated by correlating weight- and temperature-corrected growth rates for each year with the log-transformed total numbers of roach at the beginning of August (Table 3). There was no significant relationship between these variables. The same was true for the relationship between growth rate of roach and the total abundance of planktivorous 0+ fish (roach (*Rutilus rutilus*), bream (*Abramis brama*), smelt (*Osmerus eperlanus*), pikeperch (*Stizostedion lucioperca*), and perch (*Perca fluviatilis*), although abundance of planktivorous fishes varied by more than two orders of magnitude.

A significant correlation between the growth rate of 0+ roach and its main predator, piscivorous 0+ pikeperch (van Densen 1985), could not be found either.

Discussion

The primary aim of this study was to address the question whether 0-group roach in Tjeukemeer were food-limited. Food limitation can affect mortality and hence recruitment in two ways: (1) by starvation and (2) by enhanced vulnerability to predation (Cushing 1974; Backiel and Le Cren 1978; van Densen 1985). If the fish are not food-limited, recruitment of roach should be predominantly affected either by predation or by the reproductive potential of the parent stock.

A comparison of growth rates to examine feeding conditions does not seem to be a fruitful exercise unless temperature is taken into account. Despite this, the effect of temperature has not been evaluated as an independent factor in many growth studies on roach (Tsunikora 1969; Goldspink 1979; Pivnicka 1983; Tong 1984; Weatherley 1987).

Growth Model

The growth–weight relationship used (equation (4)) is well accepted (Brett 1979) and the estimated value of the exponent b of 0.56 is in agreement with the literature (Parker and Larkin 1959 and Larkin and Ayyangar 1961: 0.50–0.83 (cited by Elliott 1975a); Brett 1979, 0.61; Jobling 1983, 0.6). Fonds et al. (1985) report a higher value of 0.78 for plaice. In general, a value of 0.6 seems to be average for fish. The discrepancy between this value and the exponent assigned to weight for the maximal metabolic activity in relation to the weight (= 0.8 Winberg 1956) is discussed by Jobling (1980).

Although an exponential relationship between growth and temperature is often used in physiology, this function has two disadvantages. First, it fails to describe the sharp decline of growth rates at temperatures higher than optimum. Second, the weight loss observed at extreme low or high temperatures (Elliott 1975a,b) is ignored. Our data do not warrant rejecting a simple, linear function. The value of $c=11.5$°C (equation (5)), indicating the temperature at which growth in roach stops, is in agreement with the observations of Kempe (1962, cited by Muller and Meng 1986), Mann (1973); and Muller and Meng (1986).

Application of the Model

With our growth–model, variation in growth between years in Tjeukemeer (Fig. 3A) is largely explained, indicating that temperature is a major factor affecting growth of roach in eutrophic Tjeukemeer. This conclusion corroborates the conclusions for other young-of-the-year cyprinid fish Broughton and Jones (1978) and Muller and Meng (1986). However, Cryer et al. (1986) and Wieser et al. (1988) observed that growth was limited by food. Because the latter authors presented basic data, including temperature, we calculated the food parameter (Table 2) for Seefeld and Pitburg, two oligotrophic mountain lakes. The estimated value was only about half that in Tjeukemeer (Table 2). This may be related to the density of planktonic crustaceans in Seefeld which was about 20 times lower than in Tjeukemeer (June–August: $\approx$1000 individuals$\cdot L^{-1}$). For Stophenreuth (Wieser et al. 1988) the food parameter was equally low and the fact that the fish grew to a larger size can be explained by the higher temperatures.

Observed Sizes and Model Deviations

Growth observed in Tjeukemeer deviated from that predicted on the basis of laboratory experiments (Fig. 3A). The food parameter in Tjeukemeer was on average 20% higher than it was in the laboratory, and even higher between the onset of growth up to 1 August (29%). The slope of the regression line for the summer observations was greater than 1 (Fig. 3A), indicating an unexpectedly fast growth in warm years.

Since growth rates observed in the field were higher than expected from laboratory experiments carried out at excess food, a density-dependent growth in the field is not likely. Indeed, the food parameter and the abundance of planktivorous fish were not related. In reality the apparent growth in the lake might be overestimated because the observed mean size may be biased by selective predation on small individuals. This is supported by the results of predation experiments of 0+ pikeperch on 0+ cyprinids (W. M. Mooij, unpubl. data), but the absence of a significant relationship between the abundance of predators and the weight and temperature corrected growth rate in August would suggest that size biased predation is not the only cause of the discrepancy.

Lengths of 0+ roach were estimated from catches in the central zone of the lake. The littoral zone, where the smallest individuals are found (W. M. Mooij, unpubl. data), was not sampled. One would expect differences in size distribution in the lake to be stronger in summer than in autumn, explaining the differential bias between summer and autumn. Although

data are limited, there does not seem to be a difference in water temperatures between the two zones. Overestimation of the lengths in August could be due to the fact that smaller specimens may escape through the meshes of the sampling nets, but this effect would be expected to be stronger in the colder years when fishes are relatively small. In fact, the steeper slope would rather indicate an opposite effect.

Other possible explanations for the observed differences relate to the rearing experiments. Limited space, stress from outside the aquaria, and irregularities in the availability of food may have resulted in suboptimal growth rates. Also the fact that frozen food had a direct effect on growth rate indicates that quality and type of food can play a significant role. Line B in Fig. 1B represents the growth–temperature relationship that follows from the value of the food parameter of Tjeukemeer ($a = 4.2 \cdot 10^{-3}$, results in Fig. 3B and line B in Fig. 4). From Fig. 3B it is clear that this assumption about the value of a_{max} does not explain the steeper slope of the regression lines of the faster observed growth during summer.

Despite uncertainties, our analysis of growth data for roach indicates that growth in Tjeukemeer may be close to the maximum for roach. The high rates of growth are supported by the high densities of planktonic crustaceans present in Tjeukemeer during summer. Further evidence in support of the view that growth of roach in Tjeukemeer is high was obtained by comparing our results with published growth and temperature data from other environments. In this respect our model may be a useful tool for comparison of growth rates, corrected for temperature and weight influences, for fish under different conditions.

Acknowledgments

The project was originally suggested and stimulated by professor J. J. Zijlstra. Unfortunately professor Zijlstra passed away recently and thus could not bear witness to the final outcome of this study. Moreover we thank the following people: Eddy Lammens and Wim van Densen for providing the field data; the Organisation for Improvement of Inland Fisheries, in particular Frans Samuels, for help in the rearing experiment; Anja van der Honig for typing both data and text; Maarten Boersma, professor Niels Daan, Ramesh Gulati, Eddy Lammens and Koos Vijverberg for commenting on the manuscript.

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APPENDIX: Results of the laboratory experiments. Four observations (*) were not used for estimating the model parameters because the animals were transported from one to another temperature at the beginning of this period. Another four observations (**) were not taken into account because as a result of feding with frozen zooplankton there was a sharp decline of growth rates during this period. na = data not available.

Days after hatching	Mean water temperature (°C)	Mean freshweight (mg)	mean forklength (mm)	SD lengths (mm)	Number measured fishes	
11	15.2	2.2	8.0	0.34	25	
19	15.7	5.7	10.6	0.65	23	
26	16.2	11.8	12.4	0.49	12	
33		22.6	15.2	0.76	23	
33	na	22.6	15.2	0.76	23	*
40	11.8	32.1	16.3	1.25	9	
47	13.3	36.4	16.2	1.11	22	
57	13.3	51.9	19.3	0.93	15	
67	13.3	65.4	20.1	0.94	10	
81	13.5	83.6	21.8	1.40	10	**
128	13.4	144	27.1	1.36	8	
178		397	34.4	1.87	19	
33	na	22.6	15.2	0.76	23	*
40	17.1	36.9	17.1	0.50	15	
47	19.1	52.9	18.2	1.52	27	
57	20.1	130	23.3	2.93	15	
67	19.4	284	30.4	2.03	17	
81	18.7	403	34.1	2.33	12	**
128	16.4	1190	48.8	2.44	8	
178		2810	62.1	3.96	15	
33	na	22.6	15.2	0.76	23	*
40	20.3	37.8	17.2	1.25	10	
47	20.3	87.0	21.2	1.10	25	
57	21.5	217	27.0	1.06	16	**
67	20.7	459	35.2	1.11	11	
81	20.2	667	39.4	1.58	8	
128	20.3	2840	62.4	3.26	5	
178		6020	78.0	4.12	8	
33	na	22.6	15.2	0.76	23	*
40	24.4	45.2	17.9	0.92	13	
47	24.1	135	23.7	1.18	22	
57	24.1	391	32.2	1.36	20	
67	24.0	687	39.7	1.76	17	
81	24.2	849	43.1	1.92	10	**
128	24.6	4500	70.0	1.22	4	
178		8500	87.0	2.29	8	

Temporal Changes of Allele Frequency in Pacific Salmon: Implications for Mixed-Stock Fishery Analysis

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Waples, R. S. 1990. Temporal changes of allele frequency in Pacific salmon: implications for mixed-stock fishery analysis. Can. J. Fish. Aquat. Sci. 47: 968–976.

The effects of temporal variation in allele frequency on mixed-stock fishery analysis of Pacific salmon (*Oncorhynchus* spp.) are examined. The concept of effective sample size (S_e), which equates the precision obtained from a sample from a finite population with that from one with no temporal variability, is used to evaluate the magnitude of the problems introduced by genetic drift. Results from simulations modeling the overlapping year-class pattern typical of chinook salmon (*O. tshawytscha*) indicate that the ratio of effective to actual sample size (S_e/S) is determined primarily by the ratio of sample size to the effective number of breeders per year (S/N_b). Unless N_b is large relative to S, effective sample size can be considerably less than the actual number of individuals sampled. Sampling in more than 1 yr results in a higher S_e than does taking the same total number of individuals in 1 yr; furthermore, the advantages to multiple sampling are greatest in small populations, in which the effects of genetic drift are most pronounced. By choosing an appropriate sampling strategy, the sources of uncertainty in the analysis attributable to genetic drift can be reduced below any arbitrary level.

Les effets des variations temporelles dans la fréquence des allèles dans l'analyse des pêcheries de stock mixte de saumon du Pacifique (*Oncorhynchus* spp.) sont examinés dans ce rapport. La notion de taille d'échantillon efficace (S_e), qui permet d'obtenir la même précision à partir d'un échantillon de population finie qu'à partir d'un échantillon de population n'affichant aucune variabilité temporelle, est utilisée pour évaluer l'ampleur des problèmes introduits par la dérive génétique. Les résultats obtenus grâce à la modélisation de simulation des configurations de classe d'âge chevauchante typique du saumon quinnat (*O. tshawytscha*) indiquent que le ratio de la taille de l'échantillon efficace et de la taille de l'échantillon réel (S_e/S) est déterminé avant tout par le ratio de la taille de l'échantillon et du nombre efficace de reproducteurs par année (S/N_b). À moins que N_b soit important comparativement à S, la taille de l'échantillon efficace peut être considérablement inférieure au nombre réel d'individus échantillonnés. Un échantillonnage sur plus d'un an donne des valeurs de S_e plus élevées que le relevé du même nombre total d'individus en un an; en outre, les avantages de l'échantillonnage multiple sont plus importants dans les petites populations, dans lesquelles les effets de la dérive génétique sont très prononcés. En choisissant une bonne stratégie d'échantillonnage, les sources d'incertitude dans l'analyse attribuables à la dérive génétique peuvent être réduites sous un quelconque niveau arbitraire.

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Effective management of fishery resources is difficult even for individual, well-defined stocks, and the problem rapidly increases in complexity if multiple stocks contribute to a fishery. Such is the case with Pacific salmon (*Oncorhynchus* spp.), which spawn in discrete freshwater populations but generally are harvested at sea, where individuals from populations thousands of miles apart may be taken in the same catch (e.g. Utter et al. 1987). Determining the proportional representation of different populations in the fishery is important for a number of reasons: to determine the extent to which resources originating from one nation or state are harvested by another; to monitor the effectiveness of hatchery supplementation programs; to assure adequate escapement of particular stocks for conservation purposes or to meet treaty quotas.

A variety of approaches has been used to achieve these goals, but many recent applications have focussed on one technique in particular — the maximum likelihood mixture model developed by Milner et al. (1981). Some variations of this method have been proposed (e.g. Fournier et al. 1984; Millar 1987; Pella and Milner 1987), but all share certain features. A suite of characters is measured in fish from a mixture and in baseline samples from stocks potentially contributing to the mix; the maximum likelihood estimates of stock composition are those that maximize the probability of observing the array of character states found in the mixture, given the frequencies of those same character states in the contributing stocks.

Although many types of characters can be used in the analysis (e.g. scale patterns, meristics, parasites), most applications use genetic markers determined by protein electrophoresis (Grant et al. 1980; Miller et al. 1983; Beacham et al. 1985a,b; 1987; Utter et al. 1987). This approach, often termed Genetic Stock Identification (GSI), has several advantages: (1) a large number of potentially useful genetic markers (gene loci) can be surveyed relatively easily; (2) expression of the markers is generally unaffected by environmental conditions; (3) the genetic marks are intrinsic to the populations and are passed on to the next generation.

Regardless of the type of characters used, the mixture models make some simplifying assumptions to render a complex problem more tractable. First, the entire mixture is apportioned among the stocks identified as potential contributors. Resulting stock composition estimates will be biased if some contributing

stocks are not represented by baseline samples. Second, frequencies of the characters in the baseline samples are treated as parameters (known without error), whereas in reality they are merely estimates of population frequencies.

The maximum-likelihood algorithms currently used in GSI analyses do not deal explicitly with violations of either of these assumptions, although the potential problems have been recognized. The importance of sampling error has been evaluated by simulation (Wood et al. 1987), and bootstrap methods proposed by Fournier et al. (1984) have been used to estimate the magnitude of error from this factor (Beacham et al. 1987). A modified mixture model developed by Smouse et al. (1990) directly addresses both of the above mentioned limitations of the current model. Their model explicitly treats sample frequencies as estimates and allows a test of the hypothesis that unsampled stocks contribute significantly to the mixture.

There is, however, an additional source of error in the analysis that has not received any comprehensive treatment: the temporal stability of population allele frequencies. Blouw et al. (1988) stressed the importance of considering intrapopulational variability in any stock identification program. The problem posed by temporal variability of allele frequencies is that even large baseline samples may not satisfactorily describe population frequencies if those frequencies change over time. Fish entering the mixture will typically be drawn from different (or more) years than have been sampled; errors may result because the baseline sample provides incomplete information about allele frequencies in the array of breeding populations contributing to the mix. This is a particular concern for mixed-stock fisheries of Pacific salmon, because these species are semelparous (100% turnover in spawning populations each year) but individuals from a single brood year may return to spawn at a variety of ages.

Several approaches for dealing with this problem are possible. Temporal variation can be ignored entirely, under the assumption that differences within populations will be small compared with those between populations. At the other extreme, multiple samples can be taken from each contributing population, corresponding to the brood years appearing in the fishery. Some researchers have combined allele frequencies from temporally-spaced samples, either routinely (Beacham et al. 1985a, 1987) or conditionally, provided no statistically significant frequency differences are found (WDF 1988).

There are drawbacks to each of these approaches. Waples and Teel (1990) showed that year-to-year changes in allele frequency due to drift may be non-trivial even in relatively large Pacific salmon populations. Therefore, simply ignoring temporal change is not appropriate. On the other hand, gathering baseline samples from every relevant year class in all populations contributing to a mixture is generally not a realistic alternative. Increasing the number of baseline samples two- or three-fold also complicates the analysis and increases the possibilities for bias. Furthermore, such as a tactic fails to utilize one of the main advantages of genetic marks — their heritability.

The purpose of this paper is to provide a framework for evaluating the importance to GSI analyses of temporal changes caused by genetic drift. As shown below, another option, combining frequencies in samples from different years, has the advantage that by choice of an appropriate sampling scheme, the additional source of error created by genetic drift can effectively be eliminated. A standard statistical test, however, is not an appropriate tool to use in determining whether to combine temporally-spaced samples for mixed-stock fishery analysis.

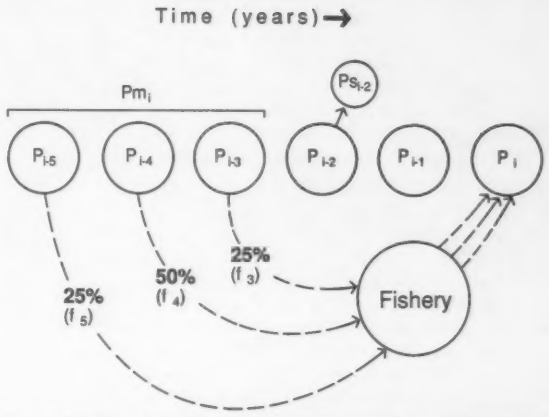

FIG. 1. Model used in the simulations. The spawning population each year (top row of circles) consists of N_b effective breeders. Most Pacific salmon species spawn at a variety of ages, so a typical breeding population might be made up of fish from brood years $k = 3, 4, 5, \ldots$years before, in the proportions f_k. In the basic model (shown), the spawning population in year i is composed of 50% 4-yr-old fish (brood year $i-4$) and 25% each 3- and 5-yr-olds. Allele frequencies are denoted by P_i (population frequency in year i), Ps_i (frequency in baseline sample from brood year i), and Pm_i (mean frequency in brood years contributing to fishery in year i). The problem posed by temporal variability arises because, in general, population frequencies in the year sampled (year $i-2$ in the example shown) are different from those in the brood years contributing to the fishery (P_{i-3}, P_{i-4}, P_{i-5}).

The Model

The following model incorporates life history features that are typical of many salmonid species (Fig. 1). Consider a population with N adults returning to spawn each year, and assume that the effective number of breeders (N_b) is the same as the actual number. (This assumption will later be relaxed.) Mutation, selection, and migration from other populations are assumed to be unimportant. Age at return varies in most Pacific salmon species, so the breeding population in year i may be composed of individuals spawned in years $i-3$, $i-4$, etc. The fish surviving to spawn are a subset of those exposed to the fishery in that year. A baseline sample (or samples) of S individuals for genetic analysis is also taken, typically one or more years prior to the fishery of interest. Allele frequencies (say, for allele A at a particular locus) can be designated as follows: in year i, let P_i be the population frequency, let Ps_i be the sample frequency, and let Pm_i be the mean allele frequency in the brood years producing fish for the mixture (e.g. in the example shown in Fig. 1, a mixture in year i is made up of fish from brood years $i-3$, $i-4$, and $i-5$).

This model provides a means of quantifying the effects of temporal variability in population allele frequencies. Consider a mixture in year i and a baseline sample taken in year $i-k$ (i.e. k years prior to the fishery; see Fig. 1). A potentially important source of error in mixed-stock fishery analysis is the difference in allele frequency between the baseline sample and the brood years producing fish for the fishery (i.e. the quantity $(Pm_i - Ps_{i-k})$). Expressed as follows, this source of error can be seen to have two components: $(Pm_i - Ps_{i-k}) = (Ps_{i-k} - P_{i-k}) + (Pm_i - P_{i-k})$. The first component $(Ps_{i-k} - P_{i-k})$ is due to random error in drawing the baseline sample, whereas the second $(Pm_i - P_{i-k})$ is caused by temporal

variability, or change in P_i over time. In the following, we will primarily be concerned with the variance of the quantity $(Pm_i - Ps_{i-k})$ (denoted by $V(Pm_i - Ps_{i-k})$). The magnitude of the temporal variability problem can be evaluated by establishing a point of reference — in this case, an appropriate point of reference would be a system in which there is no genetic drift (i.e. a population of infinite size). With this "ideal" system as a benchmark, we can ask the question, "How much precision is lost in a realistic situation, given that some temporal change in allele frequencies is inevitable?" This question can be answered by comparing $V(Pm_i - Ps_{i-k})$ for a realistic situation with that expected in an inifinite population.

In our drift model, P_i in an infinite population is the same every year (call this constant frequency P), so the second component above is 0 $((Pm_i - P_{i-k}) = (P - P) = 0)$. Thus, with no temporal variability, $(Pm_i - Ps_{i-k})$ reduces to $(Ps_{i-k} - P_{i-k}) = (Ps_{i-k} - P)$, and $V(Ps_{i-k} - P) = P(1-P)/2S$, which is just the binomial variance in choosing a baseline sample of S individuals ($2S$ genes). It is difficult to obtain $V(Ps_{i-k} - Pm_i)$ analytically for finite Pacific salmon populations, so this quantity was obtained by simulation (described below). $V(Ps_{i-k} - Pm_i)$, measured in the simulations for various values of S and N_b, can be equated with the corresponding formula for $N_b = \infty$ to find the sample size required to make the variances equal:

$$\text{(1a)} \qquad V(Ps_{i-k} - Pm_i) = \frac{P(1-P)}{2S_e} \; ;$$

$$\text{(1b)} \qquad S_e = \frac{P(1-P)}{2V(Ps_{i-k} - Pm_i)} \; .$$

The quantity S_e can be interpreted as the effective sample size, analogous to the concept of effective population size (Wright 1938). That is, a sample of size S from an actual population provides the same degree of precision as would a smaller sample (size S_e) drawn from a population with no temporal variability. In an infinite population, $S_e = S$, but effective sample size will always be less than the actual sample size if N_b is finite. Therefore, we will focus on factors that affect the magnitude of S_e.

In the model, I have ignored an additional source of uncertainty in mixed-stock fishery analysis: random error in choosing a sample from the mixture for genetic analysis. This source of uncertainty applies to infinite as well as finite populations and is not directly related to temporal variability in population allele frequencies. None of the basic conclusions reached in this paper are dependent on the size of the mixture sample, but this parameter does have some effect on absolute values of S_e. As mixture sample size decreases, a larger proportion of total uncertainty in the analysis is caused by sampling from the mixture, and the ratio S_e/S increases. Therefore, values of S_e reported here should be regarded as minimum estimates.

Computer Simulations

A series of computer simulations were performed based on the above model. I considered the frequency of one allele (allele "A") at a single locus in one population. Although multiple loci are typically used in mixed-stock fishery analysis, a single locus is sufficient here because in a pure drift model, all of the factors affecting S_e(N_b, S, k, and age structure) are the same for every locus.

Selection of the N_b breeders each year was a two-step process. First, the age of each spawner in year i was determined by a random variable, in such a way that the individual was age k with probability f_k ($\Sigma f_k = 1$). Next, the two genes carried by the spawning individual were chosen in a pair of binomial draws, with the probability of an "A" allele determined by the allele frequency (P_{i-k}) in the breeders in year $i-k$ (the brood year for the individual in question). For each year i, the fish in the mixture and those returning to spawn were presumed to come from the same array of prior brood years, so Pm_i was calculated as the weighted mean of the allele frequencies in the relevant brood years: $Pm_i = \Sigma f_k P_{i-k}$.

The baseline samples were chosein in two different ways, corresponding to procedures commonly used in studies of Pacific salmon. Adult samples, whether from hatcheries or wild stocks, are generally taken from fish that have already spawned, so adult samples in the simulations were chosen by sampling without replacement from the $2N_b$ genes in the breeding population, which was formed of fish from different brood years as described above. Baseline samples of juveniles are generally taken from the large pool of offspring resulting from the previous year's spawn. Consequently, juvenile samples for brood year i in the simulations were chosen binomially from the spawning population in year i (probability P_i of an "A" allele at each draw).

The species of Pacific salmon differ in age at spawning return. Here, I consider patterns typical of chinook salmon (*O. tshawytscha*), but the results are relevant to other species having broad overlap of year classes. In the basic model (see Fig. 1), an average of 50% of the adults return to spawn at age 4 (i.e. $f_4 = 0.5$) and 25% each at ages 3 and 5. In this model, then, the population experiences a "migration" rate (m) of 0.5 between year-classes (the proportion 0.5 "migrating" from year-classes other than the one exactly 4 yr before). Values of m from 0.33 to 0.67 were also considered.

Monte Carlo methods were used to simulate this pattern of overlapping year-classes. To initialize the simulations, allele frequencies in the N_b breeders in year-classes $i = 1$ to 5 were set to 0.5. Beginning in year 6, allele frequencies in the breeders were determined by sampling from year-classes up to 5 yr previously, as described above. This procedure was followed for 50 yr to allow the system to reach a dynamic equilibrium. Beginning in year $i = 51$ and continuing through year 100, the difference in allele frequencies ($|Pm_i - Ps_{i-k}|$) was computed for mixtures in the current year and baseline samples taken $k = 1, 2, 3, 4, 5, 10$, and 25 yr previously. This models a realistic sampling program, in which baseline samples are typically taken prior to the mixture analysis. After year 100, allele frequencies were reinitialized (to avoid gene extinction) and the process repeated. One hundred such replicates were performed for each set of initial parameters, with data taken over the last 50 yr in each replicate. Thus, 5000 data points ($|Pm_i - Ps_{i-k}|$) were available for each value of k years separating a baseline sample and the mixture it might be used to analyze. The variances of these differences in allele frequency ($V(Pm_i - Ps_{i-k}) = E(Pm_i - Ps_{i-k})^2$, where E indicates the expectation) were computed over the 5000 data points and used to calculate effective sample size (S_e) as described above. In the latter calculation, parametric $P(1-P)$ was estimated by the mean of the $P_i(1-P_i)$ in the simulations.

Data were recorded from the last 50 yr in each replicate to allow time for the system to reach an equilibrium between genetic drift and "migration" between year-classes. In practice, the high percentage of "migrants" used in the simulations caused the system to equilibrate very rapidly. Simulations using

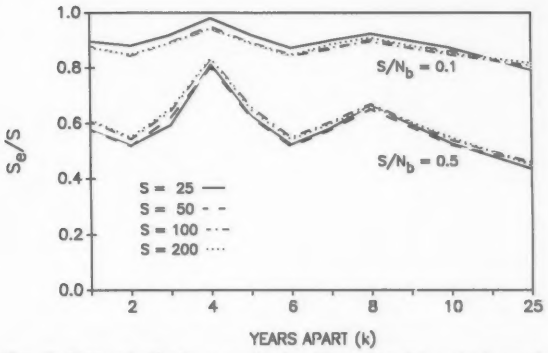

FIG. 2. Ratio of effective sample size (S_e; see text) to actual sample size (S) for baseline samples of juvenile chinook salmon taken k years prior to analysis of a mixed-stock fishery. Results are dependent on the ratio of sample size to effective number of breeders per year (S/N_b). Data are from simulations with age structure as in Fig. 1 and initial allele frequency (P) = 0.5.

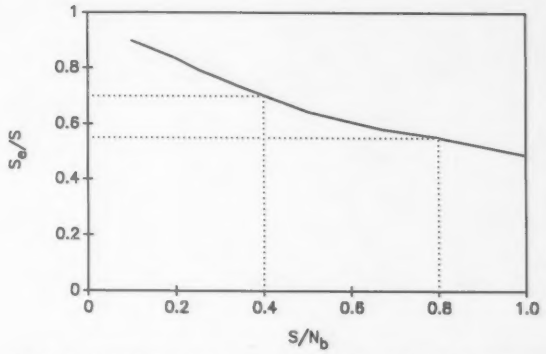

FIG. 3. Ratio of S_e to S as a function of the ratio of S to N_b for juvenile samples of chinook salmon. Results (solid line) are means for years k = 1 to 5 for simulations with S = 25, 50, 100, and 200 and a range of N_b values such that S/N_b = 0.1 to 1. In the simulations, P = 0.5 and age structure was as in Fig. 1. Dotted lines illustrate example discussed in the text: for juvenile samples, if S/N_b = 0.4, effective sample size is about 0.7 of actual sample size, whereas S_e is only about 0.55 of S if a larger sample is taken (S/N_b = 0.8).

widely divergent initial allele frequencies (0.1–0.9) in years 1 to 5 did not differ in a systematic way from those with equal allele frequencies.

Results

Most of the results that follow are for juvenile baseline samples. Results for adult samples are more complicated because they depend on the relationship between the effective number of breeders (N_b) and the total number of adults in the population (N). The relationship between adult and juvenile samples will be discussed at the end of this section.

Because several variables that can affect the ratio S_e/S were being considered (sample size, effective number of breeders, age structure, elapsed time in years between baseline sample and mixture analysis), it is difficult to illustrate all the results in simple two-dimensional plots. Fortunately, some parameters can be combined and others have relatively little effect on the analysis, as can be seen by examination of Fig. 2. This figure, which plots S_e/S for juvenile samples of various sizes taken 1 to 25 yr prior to the mixture, can be interpreted as follows. With sample size held constant, the ratio S_e/S changes with N_b (e.g. for S = 200, S_e/S is described by the lower dotted line if N_b = 400 (S/N_b = 0.5) and by the upper dotted line for N_b = 2000 (S/N_b = 0.1)). Conversely, with N_b held constant, the ratio S_e/S changes with sample size. Note, however, that although S_e/S is strongly dependent on both S and N_b, it is not their absolute magnitude that is important, but rather their ratio (S/N_b). Thus, as seen in Fig. 2, S_e/S is very nearly the same for four different values of S, provided that N_b also changes in proportion to S. A similar result was found for adult samples. In most subsequent plots, therefore, the ratio of effective to actual sample size is expressed as a function of S/N_b, the data presented being the means for all simulations with the same ratio S/N_b.

Figure 2 also indicates that except for samples taken exactly 4 yr prior to the fishery, effective sample size is not strongly dependent on the timing of the baseline sample relative to the sample from the mixed-stock fishery. This is particularly true if the breeding population is much larger than the sample. The peak for S_e at year 4 is a consequence of using in the model a fixed probability of 0.5 of return at that age; allele frequencies in a baseline sample taken exactly 4 yr previously are expected to be most strongly correlated with those in the mixture because half of the fish in the fishery are progeny of that same breeding population. In contrast, samples taken (for example) 2 yr before a mixture analysis are not quite as valuable because in the model age 2 fish are not yet in the fishery. In reality, however, age 2 males (jacks) and fish over 5 yr of age return to spawn in some chinook salmon populations, and the probability of return at ages 3, 4, and 5 is unlikely to remain constant every year. Data for many real chinook salmon populations would probably appear as a flatter curve than that shown in Fig. 2. In most subsequent plots, pooled results for baseline samples taken 1 to 5 yr before the mixture analysis are shown. Another important result shown in Fig. 2 is that S_e/S decreases as S/N_b increases (e.g. from S/N_b = 0.1 to 0.5). This result can be understood by considering a fixed sample size S. A smaller ratio S/N_b implies larger N_b (therefore less temporal variability due to genetic drift), and precision more nearly approaches that obtained for an infinite population. Conversely, a larger ratio S/N_b implies that the number of breeders is relatively small, so the uncertainty introduced by genetic drift makes a proportionally larger contribution to $V(Ps_{i-k} - Pm_i)$, and effective sample size decreases.

Given that S_e/S will be less than unity for any sample taken from a finite breeding population, what can be done to maximize S_e and hence minimize the source of error attributable to temporal variation? To illustrate the problem, consider a juvenile sample of size 100 from a breeding population of 250. From Fig. 3, S/N_b = 100/250 = 0.4 implies that $S_e/S \approx 0.7$, so the effective size of this sample is only about 70. Is it possible to increase S_e to the original sample size (100), or nearly so? One approach is to take a larger baseline sample. Figure 4 plots the relationship between S_e/N_b and S/N_b (i.e. for a given N_b, the change in effective sample size as actual sample size increases). The slope of the curve is positive, indicating that taking a larger sample increases effective sample size and will improve precision. However, S_e does not increase linearly with S, as would occur in an infinite population (broken line in Fig. 4). Rather,

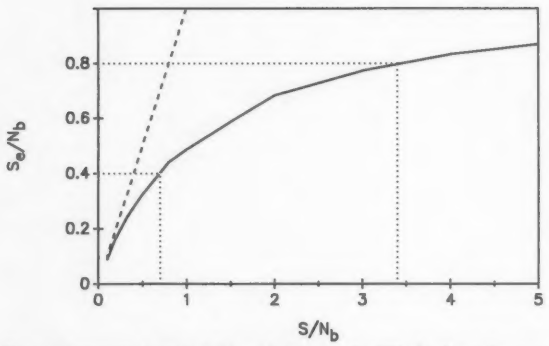

FIG. 4. Increase in ratio S_e/N_b with increase in S/N_b for juvenile samples of chinook salmon. Broken line (constant slope) indicates relationship in a population with no genetic drift; therefore, magnitude of reduction in S_e due to finite population size is an indication of the potential severity of the problems introduced by temporal variability. Results are means for years $k=1$ to 5 for simulations with $N_b=25$, 50, 100, and 200 and a range of sample sizes such that $S/N_b=0.1$ to 5. In the simulations, $P=0.5$ and age structure was as in Fig. 1. Dotted lines illustrate example discussed in the text, with N_b presumed to be 250. If juveniles are sampled and an effective sample size of $S_e=100$ is desired (i.e. $S_e/N_b=0.4$), this can be achieved by sampling enough individuals to make $S/N_b \approx 0.7$, or about 175 fish. An effective sample size of $S_e=200=0.8\,N_b$ can be achieved from a juvenile sample only by sampling about 850 fish ($S/N_b \approx 3.4$, $S=3.4\times250$).

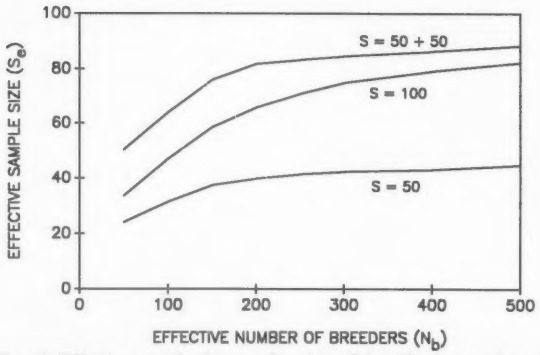

FIG. 5. Effective sample size as a function of the effective number of breeders for three sampling strategies: (a) a single sample of $S=50$ individuals; (b) a single sample of 100 individuals; and (c) two samples of 50 individuals each taken 1 yr apart. Two samples of 50 each are "worth" more (higher S_e) for mixed-stock fishery purposes than a single sample of 100, and the difference is greater in smaller populations. Results were obtained by simulation and are means for years $k=1$ to 5 for juvenile samples of chinook salmon. In the simulations, $P=0.5$ and age structure was as in Fig. 1.

for a given N_b, larger samples yield ever smaller increments of effective sample size.

Since the slope of the S_e:S curve is less than unity, doubling (say) the size of the baseline sample will not increase effective sample size by a factor of 2. The magnitude of the increase in S_e depends on the size of the breeding population; the proportional increase is smaller if S/N_b is large (Fig. 4). To consider the above example (with $N_b=250$), doubling S from 100 to 200 (i.e. from $0.4\,N_b$ to $0.8\,N_b$) increases S_e only by roughly 50%, from about 70 to about 110 ($S_e/S \approx 0.55$, $S_e \approx 0.55\times200$; cf. Fig. 3). That is, with a breeding population of effective size 250, a sample of 200 juveniles provides the same degree of prevision in estimating allele frequencies in the mixture as would a sample of 100 from a population with no temporal variability.

The problem can be approached from a different direction by referring to Fig. 4 and asking the question, "Assuming we want precision equal to that for a sample of 100 fish from a population with no temporal variability, how much larger a sample must be taken from a finite population to achieve $S_e=100$?" The required sample size can be found by drawing a horizontal line at the desired S_e/N_b value and reading S/N_b at the point of intersection with the sampling curve. In the above example, it is possible to achieve $S_e=100$ by approximately doubling the actual sample size, but this will not always be the case for an arbitrary S_e that is deemed suitable. For example, if $S_e=200$ is desired for the above population, we require a sample size that will make $S_e/N_b=200/250=0.8$. As shown in Fig. 4, this would require an actual sample of about 850 juveniles ($S/N_b \approx 3.4$; $S=3.4\times250$). Because the curve flattens out rapidly for sample sizes larger than the effective number of breeders, very large increases in sampling effort are required for any significant increase in precision. In the limit (S infinite), a juvenile sample provides a perfect estimate of population allele frequencies in one year, but even this (impossible) sampling effort is not sufficient to increase S_e above any arbitrary level. This is because a single sample estimates the allele frequencies in only one breeding population, while fish in the mixture are derived from an array of prior years.

There are limits, then, to the benefits to be derived from taking larger baseline samples. There is, however, another approach available — to take samples in more than one year and combine them to arrive at a single estimate of allele frequency. Figure 5 illustrates the advantages of this approach: S_e for two samples of 50 each taken 1 yr apart is greater than that for a single sample of 100. Results shown are means for mixtures analyzed 1 to 5 yr after the last sample was taken. Significantly, the proportional increase in S_e (relative to the value for a single sample) gained by taking two samples is greatest when the number of breeders is small — precisely the circumstances under which genetic drift is most pronounced and the potential errors arising from temporal variability the most serious. Note that for two samples taken in consecutive years, S_e is almost exactly twice that for a single sample.

In fact, the strategy of taking multiple samples provides a means of effectively eliminating the problems genetic drift creates for mixed-stock fishery analysis. Considering an ideal system (no temporal variability), there must be some sample size above which the unavoidable uncertainty in drawing the baseline sample is deemed acceptable. In their (1987) paper, Wood et al. found that baseline samples as small as 40 were effective in reducing bias and increasing precision of stock composition estimates. In practice, somewhat larger samples ($S \approx 50$–200) are often taken, but the actual number is not important here; assume that a minimum acceptable sample size is identified, and call this number S^*. Provided that it is possible to sample in such a way that $S_e \geq S^*$, the precision afforded by the baseline sample(s) will be equivalent to that desired, and the additional uncertainty in the mixture analysis attributable to genetic drift will be fully compensated for.

Figure 6 plots S_e/S^* as a function of S^*/N_b for juvenile baseline samples (each of size S^*) taken singly, as well as in pairs or triplets from differents years. If baseline data from at least

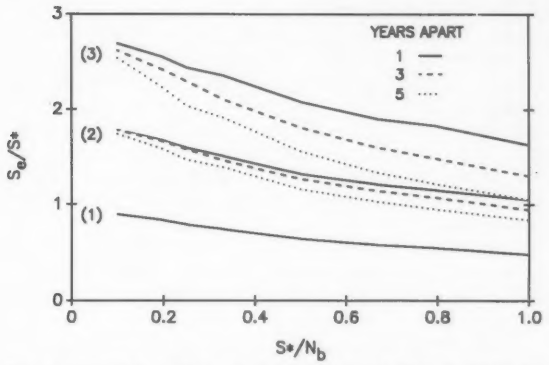

FIG. 6. Ratio of S_e to S^* as a function of the ratio S^* to N_b if baseline allele frequencies are computed by averaging data from one, two, or three temporally-spaced samples, each of S^* juvenile chinook salmon. It is assumed that S^* has been identified as the minimum baseline sample size providing an acceptable level of error in estimating population allele frequencies. By taking multiple baseline samples, it is possible to achieve an effective sample size as large or larger than S^*, thus fully compensating for the additional source of error caused by genetic drift. Sampling in consecutive years results in slightly higher S_e than if samples are taken 3 or 5 yr apart. Results are means for years $k=1$ to 5 for simulations with $S=25$, 50, 100, and 200 and $S/N_b=0.1$, 0.2, 0.25, 0.33, 0.5, 0.67, 0.8, and 1. In the simulations, $P=0.5$ and age structure was as in Fig. 1.

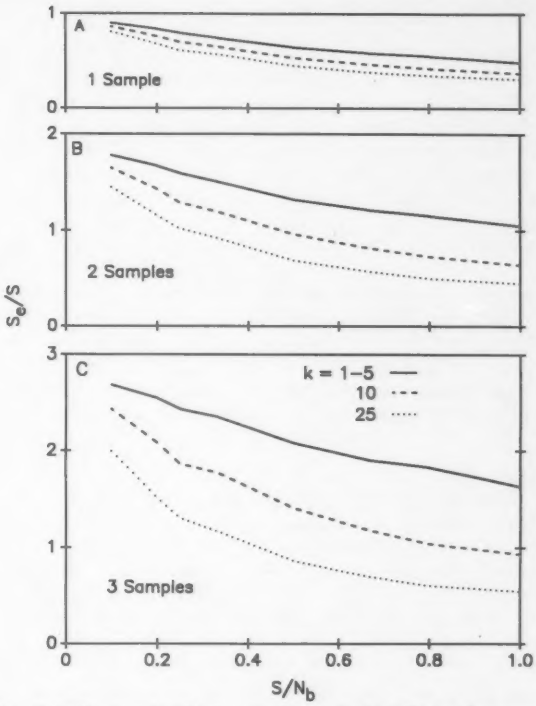

FIG. 7. Differences in effective sample size for juvenile baseline samples taken $k=1$ to 5, 10, or 25 yr prior to mixture analysis. In graphs B and C, multiple samples were taken 1 yr apart, the most recent being k years prior to the mixture. Results are means from simulations with $S=25$, 50, 100, and 200 and $S/N_b=0.1$, 0.2, 0.25, 0.33, 0.5, 0.67, 0.8, and 1. In the simulations, $P=0.5$ and age structure was as in Fig. 1.

two different years are available, effective sample size will be at least as large as the desired standard (S^*). This is true whether the samples are taken 1, 3, or 5 yr apart, although S_e is slightly higher if the samples are taken in consecutive years. Because there is no direct gene flow between Pacific salmon populations separated by a single year, sampling in consecutive years provides largely independent data regarding allele frequencies in the system as a whole. In contrast, allele frequencies in samples taken 3 or 5 yr apart are more strongly correlated and therefore contain some redundant information about frequencies in parents and their offspring.

To simplify the above analyses, the parameters P, m, and k were held constant. It is now of interest to examine results as they are allowed to vary.

Time Between Baseline Sample and Fishery

In Figs. 3–6, data for samples taken $k=1$ to 5 yr prior to the mixture were averaged because there was relatively little difference in results among those years. (In the case of multiple samples (Fig. 6 and 7), k refers to the number of years between the mixture analysis and the last sample taken). It was noted earlier (with reference to Fig. 2) that results obtained for samples taken 10 or more years prior to the mixture were not substantially different from those for years 1 to 5. This is a consequence of the pattern of overlapping year-classes in Pacific salmon. Although year-to-year variability due to drift is largely governed by the number of breeders per year, the system as a whole evolves at a rate determined by the number of breeders per generation (one generation is about 4 yr in the current model), which is a much larger number. Nevertheless, a decline in S_e was observed in the simulations if time between the last baseline sample and the mixture analysis was very large (Fig. 7). Even if nothing besides genetic drift is affecting allele frequencies, a single sample taken 10 or more years prior to the mixture will provide somewhat less precise information than will a more recent sample, and the effectiveness of multiple samples closely spaced in time also declines if they were all taken more than 10 yr prior to the mixture.

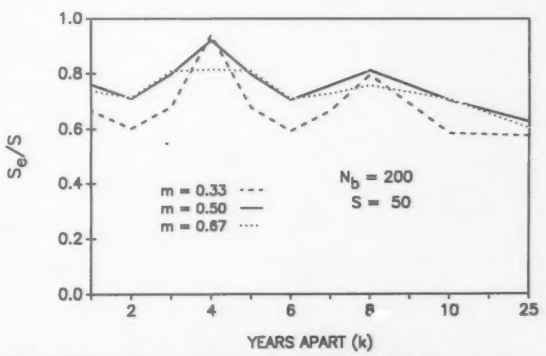

FIG. 8. Effects of age structure and pattern of overlapping year-classes on ratio S_e/S for juvenile samples of chinook salmon taken k years prior to a mixed-stock fishery analysis. Migration rate (m) is the proportion of individuals in each spawning population "migrating" from populations 3 and 5 yr before; $1-m$ is the proportion of 4-yr-old fish in the spawning population. Results are from simulations with initial allele frequencies set to 0.5 and N_b and S as indicated.

Allele Frequency

Initial allele frequencies in years $i = 1$ to 5 were set to 0.5 in all the simulations described above. Theoretically, $S_e = P(1-P)/(2V(Pm_i - Ps_{i-k}))$ should be independent of allele frequency because the term $P(1-P)$ is a factor of $V(Pm_i - Ps_{i-k})$. However, the quantity $(Pm_i - Ps_{i-k})$ may not be normally distributed if the P_i are too close to 0 or 1, particularly if S or N_b are small. Because most species used in GSI analyses have some gene loci with the common allele at relatively high frequency, it seemed prudent to examine the properties of S_e for P higher than 0.5. Results (R. S. Waples, unpubl. data) demonstrated that, even for P as high as 0.95 and S and N_b as small as 25 and 100, respectively, the estimate of S_e was virtually identical to that for $P = 0.5$. This was also true if two or three samples taken 1 to 5 yr apart were combined. Therefore, results presented here appear to be robust to deviations from $P = 0.5$ unless allele frequencies are very extreme or sample size very limited.

Age Structure

Available life history information (e.g. Nicholas and Hankin 1988), indicates that the age structure used in the above simulations (depicted in Fig. 1) is a reasonable model for chinook salmon. Nevertheless, considerable variation around this basic pattern occurs over the broad geographic range of this species, so simulations were also done using $m = 0.33$ (two-thirds of spawners returning at age 4) and $m = 0.67$ (one-third of spawners in each age class 3, 4, and 5). Results for $m = 0.67$ (even age distribution) were almost identical to those for $m = 0.5$, except for the absence of a peak for samples taken exactly 4 yr prior to the mixture (Fig. 8). If the percentage returning at age 4 was as high as 67% ($m = 0.33$), S_e was noticeably higher for $k = 4$ than for other values. Apparently, if a single age-class consistently makes up as much as two-thirds of each spawning population, a sample taken from the "right" year can be substantially better for use in the mixture analysis. Otherwise, the exact timing of the sample is not so important.

Adult versus Juvenile Sampling

All of the above results apply to samples of juveniles. Analysis of adult samples is more complicated and depends on the relationship between the actual (N) and effective number (N_b) of spawners. The basic difference between juvenile and adult sampling can be illustrated by making the simplifying assumption that $N_b = N$. In this case, an adult sample is "worth" more (higher S_e) than a juvenile sample because the variance of an adult sample with respect to population allele frequencies ($V(Ps_i|P_i)$) is less by the factor $(1 - (2S-1)/(2N_b-1)) \approx (1 - S/N_b)$ (e.g. Sokal and Rohlf 1981):

(2a) for juveniles: (binomial sampling) $$V(Ps_i|P_i) = \frac{P_i(1-P_i)}{2S};$$

(2b) for adults: (hypergeometric sampling) $$V(Ps_i|P_i) = \frac{P_i(1-P_i)}{2S}\left[1 - \frac{2S-1}{2N_b-1}\right].$$

In large populations, S/N_b is generally small and it may make little difference whether adults or juveniles are sampled, but in small populations the difference in precision can be substantial.

Equations (2a) and (2b), however, provide an incomplete picture about precision in mixed-stock fishery analysis, for two reasons. First, the effective size of natural populations is generally thought to be smaller than the census size, in which case allele frequencies in the total and effective breeding populations will generally differ. A juvenile sample still estimates the allele frequency in the $2N_b$ genes making up the effective breeding population, with variance given by equation (2a), but an adult sample may include individuals that do not contribute to the next generation or do so to a disproportionately small degree. Thus, if $N_b < N$, the variance of an adult sample with respect to allele frequency in the effective breeding population will be greater than that given in equation (2b). Waples and Teel (1990) showed that if N_b is less than half the total number of adults subject to samping, $V(Ps_i|P_i)$ for an adult sample is greater than that for a juvenile sample of the same size from the same brood year.

In the present context, however, it is not $V(Ps_i|P_i)$ that is the error term of primary interest, but rather $V(Ps_{i-k} - Pm_i)$, which is not so easy to evaluate analytically. Because of this complication, I ran a series of simulations, using the basic model described above, to address the following question: "Assuming an option of sampling a given number (S) of juveniles or adults from the same brood year, which yields the higher S_e?" Results indicate that even if $N_b < N$, S_e is almost always higher for adult samples than for juveniles. The advantages to sampling adults are greatest if (1) N_b is nearly as large as N; (2) the sample size is a substantial proportion of the effective number of breeders; or (3) the baseline sample is taken within a few years of the fishery to be analyzed.

Discussion

Results presented here provide a means of evaluating the importance of genetic drift for mixed-stock fishery analyses. The major findings can be summarized as follows:

(1) For analyses in which the age distribution of the fishery and the spawning populations is well known, sampling in the appropriate year(s) may improve the estimates of stock composition. In general, however, for species will broad overlap of year-classes, the exact timing of the baseline samples does not strongly affect precision.

(2) For any sample of size S drawn from a finite population, effective sample size is always smaller than would be the case if there were no temporal variability, and the reduction is more pronounced in small populations.

(3) Within limits, S_e can be increased by taking a larger initial sample. However, a point is inevitably reached at which larger samples yield very small increases in precision. This point is reached very quickly in small populations.

(4) A more effective strategy than simply taking larger samples is to sample in more than one year. This strategy has the advantage that it combines genetic information from multiple spawning populations, each of which contains information pertinent to allele frequencies in subsequent years. By use of combined samples from two or more years, it is possible to reduce the uncertainty caused by finite population size below any arbitrary level. In essence, the problem of temporal change due to drift can be compensated for by choice of an appropriate sampling plan.

If sufficient information were available regarding age structure in the fishery and the source populations, a strategy for weighting the allele frequencies in the temporal samples could be formulated to account for the relative degree of precision each sample could be expected to provide. Such a weighting scheme, however, would be difficult to compute, and results

presented here indicate that the expected benefits would typically be small (because S_e is generally similar for samples regardless of the year they were taken).

(5) The advantages to combining allele frequencies in samples from different years are greatest when the ratio of S to N_b is large. In such populations, genetic drift is most pronounced, allele frequencies fluctuate most from year-to-year, and samples from a single year provide the least precision for purposes of mixed-stock fishery analysis. It is these small populations, therefore, for which it is most important to use genetic data for multiple years.

However, standard statistical tests of the equality of allele frequencies will most often find "significant" differences for temporal samples from small populations. Such tests are sometimes used to determine whether or not to pool allele frequencies from multiple samples from the same population (e.g. WDF 1988). Waples (1989) and Waples and Teel (1990) showed that the probability of a significant test comparing temporally-spaced sample is determined primarily by the ratio of S to N_b. Unless N_b is much larger than S, this probability can be many times the nominal α level, the inflation being due to changes in population allele frequency caused by genetic drift. Use of a standard statistical test will thus tend to discourage lumping samples from small populations (because of genetic drift), whereas it is small population for which it is most important to have multiple samples (because of genetic drift).

The obvious conclusion is that standard tests that do not account for drift are not appropriate for deciding whether to combine temporally-spaced samples for GSI analyses. Results presented here indicate that such samples should routinely be combined unless there is reason to believe that temporal differences are too large to be attributable to sampling error and genetic drift. A standard statistical test does not provide this information, but it still may be useful in identifying cases of substantial genetic change that may be due to factors such as migration from other populations or selection (natural or artificial). These possibilities should be carefully considered if large allele frequency shifts are observed. Results obtained by Waples and Teel (1990) provide a context for interpreting observed changes of allele frequency in Pacific salmon populations in terms of a model involving genetic drift and sampling error. The important point here is that results of standard tests should not, by themselves, be used to determine whether to combine temporally-spaced samples for mixed-stock fishery analysis.

One possible objection to combining samples from different years is that the resulting allele frequencies will be artificial and not characteristic of any biological entity. This is a valid concern in combining samples from different populations, but the situation is different with temporally-spaced samples. Combining allele frequencies in temporally-spaced baseline samples is not artificial because it mimics processes that occur in forming the breeding population each year. Consider a typical example of a mixture analysis in year i and a baseline sample from year $i-k$. Although the sample allele frequency Ps_{i-k} provides the best estimate of allele frequency in brood year $i-k$, it is not the best estimate for fish in the mixture, which come from a variety of different brood years.

(6) Because of the pattern of overlapping year-classes in Pacific salmon, long-term allele frequency changes due to drift are not substantially greater than are short term fluctuations. Samples taken many years previously continue to provide information relevant to allele frequencies in the current population. It therefore should not be necessary to continuously resample a population at intervals of a few years. However, some decline in precision is expected if the number of years separating the baseline samples and the mixture analysis is too great. Simulation results suggest that in the drift model, sampling every 5–10 yr or so should generally be sufficient to keep the estimates of population allele frequency up to date. It is important to note, however, that such a sampling program may not be adequate if strong selection is involved or if there has been substantial genetic exchange with other populations (as might occur in transfer of eggs among hatcheries or overplanting natural populations with hatchery fish).

(7) In most circumstances, the "value" (S_e) of an adult sample is higher than that for a juvenile sample, although the magnitude of the difference is a complex function of several variables (S, N, N_b, and timing of the baseline and fishery samples). All else being equal, this suggests that sampling of adults should be preferred to sampling of juveniles. There are, however, a number of other factors to be considered in selecting the life history stage to sample, including ontogenetic patterns of gene expression affecting resolution of some enzyme systems, the probability of obtaining a random baseline sample (of spawning adults or juvenile progeny), the ability to collect an adequate number of individuals, and logistics and cost of the collection effort.

(8) The relative insensitivity of the results to changes in age structure suggests that the conclusions are also applicable to other Pacific salmonids with broad overlap of year classes (e.g. sockeye salmon (*O. nerka*), chum salmon (*O. keta*), and steelhead trout (*O. mykiss*; formerly *Salmo gairdneri*)). The rigid 2-yr life cycle of the pink salmon (*O. gorbuscha*) produces essentially independent lineages in odd and even years, so for this species it is clearly necessary to survey allele frequencies in both lineages separately. Coho salmon (*O. kisutch*) spawn primarily at age 3 and therfore have a pattern of overlapping year-classes that is intermediate between the pink salmon and the other species. Preliminary results modeling coho salmon with 5–10% spawning at age 2 and the remainder at age 3 indicate that effective sample size may be considerably reduced unless samples are taken a multiple of 3 yr prior to the fishery (R. S. Waples unpubl. data). Whether this reduction in S_e translates into reduced precision in stock composition estimates will depend on the magnitude of the genetic differences between contributing stocks.

The conclusion that the problems posed by temporal variability in allele frequencies can be overcome by an appropriate sampling plan contrasts with results others have reported for variation in morphological characters. Blouw et al. (1988) found that temporal changes in meristic traits within Atlantic salmon (*Salmo salar*) populations were large relative to between-populations differences, meaning that baseline sample information must be gathered from the same cohort(s) that occur in the fishery. Lear and Sandeman (1980) reached a similar conclusion for scale pattern analysis of Atlantic salmon. The problems caused by temporal changes in morphological characters are componded by their unpredictability; when patterns in the variation can be identified, they are generally associated in a complex way with environmental variables, which themselves are mostly unpredictable. In contrast, genetic markers are heritable, and stochastic changes due to genetic drift can be modeled either analytically or by simulation (as in this paper). As a consequence, it is possible to predict the magnitude (if not the

direction) of allele frequency change and adopt a sampling strategy that takes genetic drift into account.

Precision and Accuracy of Stock Composition Estimates

In this paper, the problem of precision has been approached in a rather narrow sense (i.e. precision of a baseline sample in estimating allele frequencies in brood years providing fish to the mixture). This approach was useful in that it provided a context for assessing the importance of temporal variability and allowed us to evaluate the relative merits of different sampling strategies. Ultimately, however, the important question is how temporal variability affects bias and precision of actual stock composition estimates. The answer to this question depends heavily on the magnitude of the genetic differences between stocks contributing to the mixture. If interpopulational differences are large relative to temporal changes within populations, it may make little difference whether S_t/S is much less than unity; the algorithm may still be able to accurately and precisely resolve the mixture. On the other hand, if temporal differences are of the same order of magnitude as differences between populations, genetic drift may be a serious source of uncertainty in the analysis. Many practical applications of mixed-stock fishery analysis involve both types of situations: some populations are very distinct genetically, while other pairs or groups are similar enough that between-population differences are not substantially larger than temporal differences within a population. It is in these latter cases that maximizing the information content of the baseline samples is most crucial, and results presented here provide strategies for doing so.

Several approaches are available for evaluating precision of stock composition estimates obtained from genetic data. Milner et al. (1981) and others have used the conditional covariance matrix of composition estimates to obtain variance estimates, based on the assumption of asymptotic normality. Millar (1987) suggested use of the infinitesimal jackknife procedure to evaluate the covariance matrix, and Fournier et al. (1984) and Pella and Milner (1987) discussed the use of bootstrap methods to obtain an empirical distribution of composition estimates. Any of these methods can also be used just as easily with baseline samples that are a combination of samples taken in different years.

Another approach, which takes advantage of information on temporal variability contained in multiple samples from the same populations, is as follows (Jerome Pella, National Marine Fisheries Service, Auke Bay Laboratory, pers. comm.): a series of composition estimates is performed, each trial using just one of the temporal samples (selected by random draw) from each population. The advantage to this method is that variation in composition estimates over the series of trials would provide an indication of the uncertainty caused by temporal variability. The disadvantage is that this method only evaluates loss of precision from using individual yearly samples, whereas we have seen that maximum precision is obtained by combining temporally-spaced samples before the analysis. Ideally, a method for evaluating precision would use the technique that provides the best point estimate (combining temporally-spaced samples) without losing the information in the individual samples. This topic deserves further study.

Acknowledgments

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Mysis relicta in Lake Michigan: Abundances and Relationships with their Potential Prey, *Daphnia*

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Mysis relicta were sampled at a 100-m reference staton in southeastern Lake Michigan (43°N 86°40′W) from 1985 to 1989, to quantify nighttime water column abundances and to compare vertical distributions with those of *Daphnia*. Diel vertical migration produced maximum concentrations in the thermocline at night of 1 to 10 mysids · m^{-3}. Variation among replicates averaged 28% with a 3-net Tucker trawl and 19% with Puget Sound vertical closing nets. Mean areal abundances over 5 yr averaged 110 mysids · m^{-2} (SE = 20; n = 30; range = 25 to 645) based on nighttime vertical and oblique net tows at 1–3-wk intervals during summers at the reference station. Synoptic cruises from 43°N to 45°N during August indicated that densities were considerably greater offshore than inshore, and greater in the north than in the south. Hypothesized long-term changes in mysid abundances were not detected. Although *Mysis* is potentially an important predator on *Daphnia*, differences in nighttime vertical distributions reduce encounters between *Mysis* and *Daphnia* during summer in Lake Michigan, such that *Mysis* exert mortality rates on *Daphnia* of <1.5% per day; the latter are in general less than 10% of the birth rates of *Daphnia* populations, estimated from fecundities.

Des échantillons de *Mysis relicta* ont été prélevés entre 1985 et 1989 dans une station de référence du sud-est du Lac Michigan (43°N 86°40′W) pour quantifier l'abandance nocturne de l'espèce dans la colonne d'eau et comparer ses distributions verticales à celles de *Daphnia*. La migration verticale nychtémérale produisait des concentrations maximales de 1 à 10 mysidacés·m^{-3} dans la thermocline pendant la nuit. La variation entre les traits répétés atteignait en moyenne 28 % avec un chalutier Tucker à trois filets et 19 % avec des filets à fermeture verticale Puget Sound. L'abondance spatiale moyenne sur 5 ans atteignait en moyenne 110 mysidacés·m^{-2} (ET = 20; n = 30; fourchette = 25 à 645) d'aprés les traits de chalut verticaux et obliques effectués la nuit à des intervalles de 1 à 3 en été à la station de référence. Des croisières synoptiques effectuées entre 43°N et 45°N au mois d'août ont révélé que les densités étaient considérablement supérieures au large que près des côtes et plus importantes au nord qu'au sud. Aucun des changements à long terme supposés dans l'abondance des mysidacés n'a été confirmé. Bien que *Mysis* soit potentiellement un important prédateur de *Daphnia*, les différences dans la distribution verticale nocturne des espèces réduisent les possibilités de rencontres entre *Mysis* et *Daphnia* en été dans le Lac Michigan; ainsi, *Mysis* entraîne des taux de mortalité inférieurs à 1,5 % par jour chez *Daphnia*; ce pourcentage est en général inférieur à 10 % du taux de natalité des populations de *Daphnia*, estimé à partir de la fécondité.

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Mysis relicta, the opossum shrimp, is an important component of Laurentian Great Lakes food webs, acting both as an omnivorous predator on plankton (Grossnickle 1982) and as a preferred prey of planktivorous and demersal fish (Rawson 1961; Wojcik et al. 1986). Because of its suitability as fish forage, *Mysis* is one of the few zooplankton species introduced into lakes to enhance fish production (Lasenby et al. 1986). Some authors have reasoned that mysid abundances should be increasing in Lake Michigan owing to recent declines in planktivorous alewife, *Alosa pseudoharengus* (Stewart et al. 1981), thus intensifying predatory pressure on their preferred prey, *Daphnia* (Evans and Jude 1986; Scavia et al. 1986).

Our first objective was to estimate areal and volumetric abundances of *Mysis* at different spatial and temporal scales in Lake Michigan. Four time scales were chosen, (1) variation between replicate oblique trawls taken within 0.5 h of each other at a single station, and variation among replicate vertical net tows while at anchor, (2) daily variation associated with diel vertical migration (DVM), (3) seasonal variation through the summer, and (4) interannual variation. Two space scales were investigated, (1) a vertical scale of meters representing DVM, and (2) geographic variability on a scale of tens to hundreds of kilometers. Comprehensive surveys over mutiple summers permitted us to quantify these variations, compare areal density estimates by oblique tows and vertical net tows, and contrast

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our estimates with historical estimates of *Mysis* abundance in Lake Michigan.

Mysids have been identified as major agents of mortality on *Daphnia* in other lakes (Goldman et al. 1979; Murtaugh 1981). In Lake Michigan, however, previous work had suggested that *Daphnia* and *Mysis* have different vertical distributions at night, which might tend to reduce spatial overlap and predation. Consequently, we quantified the overlap between populations during our cruises to accomplish our second objective which was to estimate the predatory impact of *Mysis* on *Daphnia* populations.

Methods

Estimating abundances of *Mysis* is complicated by the animals' nocturnal diel vertical migration (DVM) behavior (Robertson et al. 1968; Beeton and Bowers 1982). Sampling strategies have relied historically on daytime epibenthic sled tows (McWilliam 1970; Reynolds and DeGraeve 1972; Morgan and Beeton 1978; Grossnickle and Morgan 1979; Malley and Reynolds 1979) or vertical plankton tows performed at night (Carpenter et al. 1974; Grossnickle and Morgan 1979; Morgan 1980; Nero and Davies 1982).

All of the studies cited above reported the density of *Mysis* populations on an areal basis. Only Beeton's (1960) study in Lake Michigan, using a Clarke-Bumpus net, estimated volumetric abundances at discrete depths. Although areal estimates of *Mysis* are satisfactory for analyzing its life history, population dynamics, and secondary production, volumetric estimates are required for studies of trophic interactions, especially density dependent relations between predators and prey.

Sampling

A reference station for plankton collections was established at 43°N, 86° 40′W, 36 km offshore from Grand Haven, Michigan, at 100 m depth. Samples were collected during seven cruise periods in 1985, six cruises in 1986, four in 1987, six in 1988, and six in 1989. Synoptic surveys of the lake during August of each year established multiple stations between 43°N and 45°N.

Zooplankton were collected with submersible pumps (1985–86), vertical closing nets (1985–89), and Tucker trawl (1985–86). The pumps were 3.2 A, 115 V (AC) submersible centrifugal pmps (ABS Robusta 201) equipped with 3.84 cm inner diameter, 4.92 cm outer diameter, clear PVC vacuum hose with corrugated cover (West Michigan Rubber and Supply Co., Muskegon, MI). Pumps delivered 1 $L \cdot s^{-1}$, and were calibrated at each sample stratum at least once per cruise. The pump was attached to a hydrographic winch and was raised at 1 m increments through 10 m strata from 40–30 m, 30–20 m, 20–10 m, and 10–0 m; it was raised at 2-m increments from 75–40 m. Samples were collected from each stratum by discharging pump effluent through a 130-μm (500 L) or 64-μm (250 L) nitex plankton net partially submerged in a 60-L plastic bucket.

Vertical net tows were performed with 1-m diameter Puget Sound closing nets (Research Nets, Bothell, WN) having either 300 or 130-μm mesh and 0.785 m^2 bridle openings. The sampling schedule conformed to the nocturnal DVM habits of *Mysis* in Lake Michigan (Beeton and Bowers 1982). Nighttime tows were performed at least 1 h after sunset, usually from 2300 to 0200 h. Daytime collections (1100 to 1400 h) were taken, but very few mysids were ever captured. Oblique tows were performed with a Tucker trawl (Research Nets) employing 300-μm aperture netting and having a 1-m^2 opening for each of three messenger operated nets. Day and night samples with the trawl were collected during the same time intervals as the vertical tows. Depth strata for trawl samples were selected according to the thermal regime on any given sampling day to represent the epilimnion, metalimnion, and from approximately 10–20 m above the bottom to the top of the hypolimnion. Oblique tows were taken perpendicular to the wind at ca. 1.5 knot ship speed to minimize collecting bias from Langmuir circulations. Volumes of water sampled by each net were estimated by digital flowmeters (General Oceanics, Miami, FL), the exact period each net was open, and depth estimates calculated from hydrographic wire angles.

The trawl was deployed with the bottom net open, and consequently some animals from shallow strata were included in our deep tows during 1985. In 1986, the open net was closed and a new net was open as the trawl reached maximum depth, permitting us to quantify abundances in the hypolimnion directly. In 1985, mysid densities in the hypolimnion were estimated indirectly from areal estimates based on the vertical tows and unambiguous estimates from upward moving trawls through the thermocline and mixed layer. Areal densities for the top two strata (by Tucker trawl) were subtracted from the estimates for total areal abundance (by vertical tow) which left the areal density in the hypolimnion for conversion to a volumetric estimate. This indirect method proved to be less ambiguous than trying to correct the number of animals in the bottom net for animals collected in the top two strata. Because of the estimation procedure in 1985, independent assessments of areal abundance via vertical net and Tucker trawl are possible only for 1986. In subsequent years, *Mysis* abundances were estimated exclusively by vertical closing nets.

Preservation and Sample Counts

Net, trawl, and pump samples were preserved by first adding carbonated water (1985–87 only) and then a sugar-formalin solution (Haney and Hall 1973). If ≤100 mysids were present in the whole sample, no subsampling was performed. Otherwise the sample was subsampled with a Folsom Plankton Splitter until >100 mysids were enumerated. Samples for *Daphnia* were concentrated to 80 mL (pump samples) or 300 mL (closing net samples) in a beaker and then subsampled in increments with an automatic pipet until >100 daphnids of each species were counted. Only total daphnids are reported here.

Tucker trawls were replicated and relative variation (*RV*) among nets was measured as follows:

$$(1) \quad RV = (X_i - X_p)/X_p$$

where

$$(2) \quad X_p = \Sigma N_i / \Sigma V_i,$$

and N_i are the raw mysid counts in each replicate net and V_i are the respective volumes sampled. X_p is the pooled mean density (*Mysis* $\cdot m^{-3}$) and X_i is an individual sample density.

Predation Rates

Predation rates by *Mysis* on *Daphnia* were estimated from the direct in situ measurements of Bowers and Vanderploeg (1982) in southern Lake Michigan. The data of Bowers and Vanderploeg suggest that predation rate (P = *Daphnia* killed

Mysis$^{-1} \cdot$ d^{-1}) is a linear function of prey density (D = *Daphnia* $\cdot$ m^{-3}) over the range of prey abundances encountered in Lake Michigan (D<7000):

$$(3) \quad P = 0.014\,D \qquad (r^2 = 0.924,\ n = 7).$$

The slope of a linear response relationship fit to the original data was significantly different from zero ($P \ll 0.01$) but the intercept was not; consequently the regression reported above was constrained to pass through the origin (i.e. zero predation on *Daphnia* when *Daphnia* are absent).

Equation (3) applies strictly only to adult mysids. Other workers (Cooper and Goldman 1980; Murtaugh 1981) have reported that juvenile mysids can eat *Daphnia*, but that they take small individuals and that they feed at reduced rates. Based on the work of Cooper and Goldman with *Mysis relicta*, we concluded that the functional response measured by Bowers and Vanderploeg could be applied to all mysids 15 mm or longer, but that smaller individuals fed at one third the rate of larger animals. Consequently, we measured the lengths of 50 mysids drawn from each of our samples; if fewer than 50 animals were present, all of them were measured. *Mysis* were blotted dry, placed on a millimeter scale, straightened by holding them with forceps, and measured from the tip of the rostrum to the tip of the telson. Measurement was by eye, with occasional verification with dissecting microscope. From these measurements we estimated the proportion of the population in each sample which was greater or less than 15 mm, and calculated total mortality rates accordingly. Although individual predation rates do not likely change in such stepwise fashion, when computing rates for entire populations it is useful to apply different mean feeding rates to different size classes of *Mysis*.

Our second method for estimating the rates of predation by *Mysis* on *Daphnia* entailed direct inspection of gut contents and identification of individual prey. Mysids collected by vertical net tow were selected as encountered and measured as above. The foregut of each animal was removed to a glass slide with a drop of glycerine and dissected apart to expose and disperse all prey debris before application of a cover slip. The entire debris mass was searched and the number of *Daphnia* present was ascertained from numbers of characteristic mandibles or postabdominal claws. Hindguts of animals captured in our night samples contained only diatoms and thus we believe the foreguts contained all animal prey consumed by *Mysis* so far that night.

Results and Discussion

Distribution and Variability of *Mysis* Populations

The DVM behavior of *Mysis* was the most conspicuous variation of the population in time and space. Daytime vertical tows and oblique trawls at the reference station usually captured no animals. Nighttime trawls (Fig. 1), however, revealed vertical distributions consistent with previous reports from Lake Michigan. This reported pattern is (1) avoidance of the warm epilimnion, and (2) concentrations often higher in the metalimnion than in the hypolimnion (Beeton and Bowers 1982). In 1985 densities ranged from approximately 1.0 to 4.0 mysids $\cdot$ m^{-3}. On 11 June the mixed layer was 8.5°C and mysids ascended into the surface waters at night. By 26 June the epilimnion had warmed to 11.3°C, and most *Mysis* remained deeper. Metalimnetic densities of 4.0 mysids $\cdot$ m^{-3} were the highest concentrations observed during 1985. This pattern, consistent with the behavior of a cold stenotherm, persisted in all subsequent samples.

Beeton (1960) measured nighttime vertical distirbutions of *Mysis* in 1954–55 near our station, and the patterns he observed were very similar in shape to those in Fig. 1, but densities in his study averaged 12.2 mysids $\cdot$ m^{-3} in the metalimnion and 1.4 mysids $\cdot$ m^{-3} in the hypolimnion. Beeton towed his Clark-Bumpus net at discrete depths, whereas we took oblique tows by Tucker trawl across depth strata. Mysids usually concentrate at the base of the epilimnion (Bowers and Grossnickle 1978), so that local densities measured at a single depth might exceed average values across strata. We averaged the densities presented by Beeton over depth intervals to make them comparable to our data but Beeton's values remain slightly higher. Beeton's areal estimates averaged 251 mysids $\cdot$ m^{-2}, while ours averaged 110 (SE = 20, $n = 30$ dates) mysids $\cdot$ m^{-2}, which indicates that mysid abundances have not increased from 1954–55 to 1985–89, and that they may have actually decreased. There were no significant differences in abundances among the 5 yr of our study (Kruskal-Wallis, $P = 0.28$).

Relative variation among replicate oblique trawls (RV) averaged 28.2% (SE = 3.0%, $n = 89$), and was not significantly different among depth strata (Kruskal-Wallis, $P > 0.9$). Trawl collections usually sampled in excess of 100 m^3 with each net.

Paired *t*-tests (Snedecor and Cochran 1972) indicated that the differences between areal abundances estimated by vertical tow and Tucker trawl (Table 1) were random ($P > 0.3$), as were differences between 300- and 130-μm nets. Although oblique Tucker trawl samples integrate horizontal patchiness and sample a much larger volume of water, vertical net tows require less time and are easier to perform in all types of weather. During 1988 and 1989, we quantified the variability of our vertical net collections by taking three or four replicate samples of total water column abundance at both reference and alternative offshore stations. The mean CV (Mean/SD) for nighttime samples at deep stations ($z \geq 100$ m) was 18.5% (SE = 2.0%, $n = 35$ replicate series). Our experience agrees with the findings of three other studies (Malley and Reynolds 1979; Grossnickle and Morgan 1979; Nero and Davies 1982) that nighttime vertical net tows provide easily obtained and precise estimates of areal mysid densities. Moreover, we find no evidence for reduced net capture efficiency with our large vertical closing nets.

The mean areal densities we measured at our reference station from 1985–89, 25 to 645 mysids $\cdot$ m^{-2}, overlap previous estimates for offshore Lake Michigan: 200–400 mysids $\cdot$ m^{-2} in 1969 (McWilliam 1970), 1–228 mysids $\cdot$ m^{-2} in 1970–71 (Reynolds and DeGraeve 1972), and 23–337 mysids $\cdot$ m^{-2} (Morgan and Beeton 1978) or 180–797 mysids $\cdot$ m^{-2} (Grossnickle and Morgan 1979) in 1976. The studies represent a variety of collection methods, sampling times and geographic locations. Our measurements, part of a continuing study, suggest first that the population has not changed radically in recent years, and second, provide a baseline by which to interpret future events.

Synoptic cruises revealed that areal densities offshore were generally greater than those at inshore stations (Fig. 2). Avoidance of warmer inshore regions of Lake Michigan by *Mysis* has been observed previously with both epibenthic sleds (Reynolds and DeGraeve 1972) and vertical net tows (McWilliam 1970; Grossnickle and Morgan 1979). However, the distribution patterns may have more to do with light avoidance than with direct thermal preference alone. Morgan

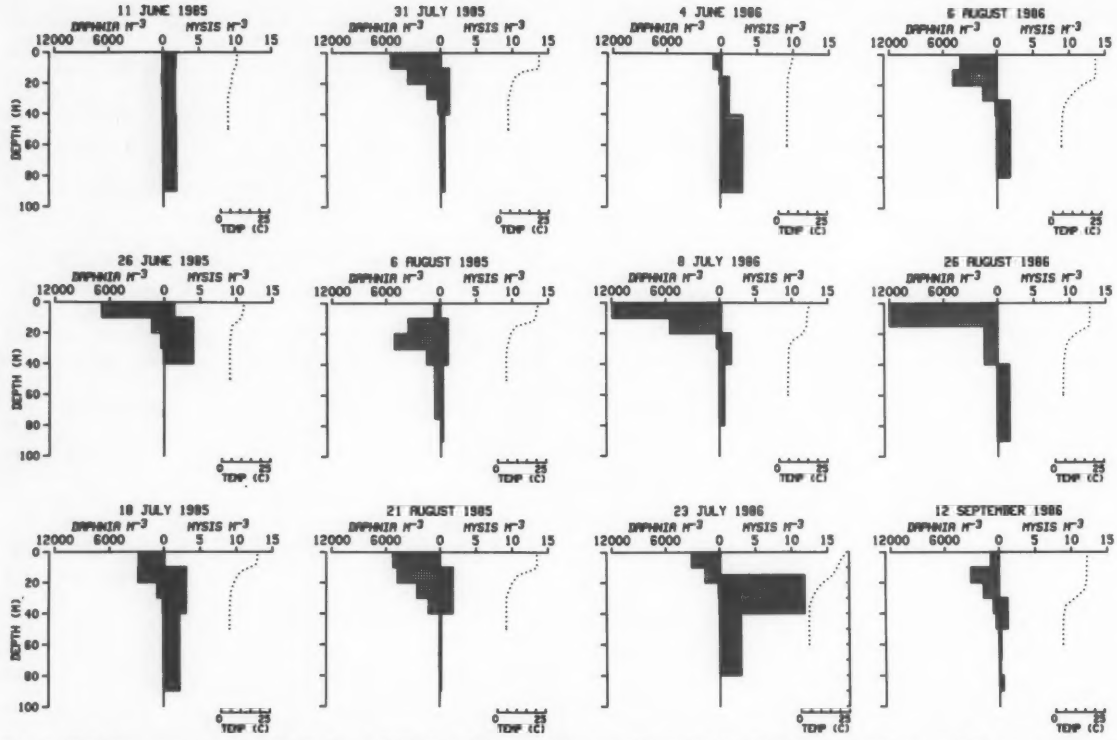

FIG. 1. Nighttime mean vertical distributions of *Mysis relicta*, *Daphnia* spp. (*D. pulicaria*, *D. galeata mendotae*, and *D. retrocurva*), and temperature at the offshore Lake Michigan reference station during 1985 and 1986. *Mysis* distributions measured with Tucker trawl and vertical closing nets; *Daphnia* distributions were measured with submersible pumps all dates except 26 August and 12 September 1986, when distributions were measured by closing net.

and Threlkeld (1982) demonstrated that young juveniles disperse horizontally to shallow waters of Lake Tahoe and later migrate horizontally towards deeper regions as they age. They hypothesized that as young mysids grow, they progressively seek deeper and darker waters during daytime because sensitivity to light increases with mysid age.

The positive correlation between water column depth and areal abundance of *Mysis* is a common pattern in the Great Lakes. Sampling with an epibenthic sled in southeastern Lake Michigan, Reynolds and DeGraeve (1972) demonstrated increased mysid densities from depths of 9 to 146 m. Carpenter et al. (1974) discovered the same pattern for Lakes Huron, Superior, and Ontario. Mysids may experience increased habitat heterogeneity in deep waters, because of reduced susceptibility to visual predators, so that some individuals can remain in the aphotic water column even in daytime, and thereby escape demersal predators. Although mysids were usually absent from daytime vertical net tows at our 100-m reference station, daytime tows at stations greater than 140 m deep routinely yielded some animals.

Mysid varations on a large horizontal scale may reflect predator–prey interactions in both the pelagic and epibenthic communities. As an omnivore, *Mysis* feeds on phytoplankton, zooplankton, and epibenthic prey (Lasenby and Langford 1973; Bowers and Grossnickle 1978; Parker 1980; Grossnickle 1982), while being at risk to planktivorous fish in the water column (Janssen and Brandt 1980) and to demersal fish on the bottom (Wojcik et al. 1986). Morgan (1980) has reported that total reproductive output of mysid populations is positively correlated to community productivity. More productive regions in the lake could support greater local mysid abundances. Our results, however, show greatest numbers of *Mysis* in the more oligotrophic northern and offshore regions of Lake Michigan. Predation by fish in different regions of the lake may more likely account for regional variations in mysid abundance than does regional variation in primary productivity.

Interactions between *Mysis* and *Daphnia*

Interannual variation in *Mysis* populations is of special interest because of recent changes in the Lake Michigan ecosystem. With increased stockings of piscivorous salmonids, planktivorous alewife (*Alosa pseudoharengus*) populations have declined (Stewart et al. 1981). In turn, the decrease of size selective predation on larger sized *Daphnia* permitted the success of larger daphnids, especially *Daphnia pulicaria* (Evans and Jude 1986; Scavia et al. 1986; Dorazio et al. 1987). These large *Daphnia*, more rapid grazers than other members of the herbivore community, were suspected to be capable of significantly reducing phytoplankton biomass and increasing water clarity (Scavia et al. 1986). Because *Mysis* is a preferred prey of the alewife and an important invertebrate predator on *Daphnia*, both Evans and Jude (1986) and Scavia et al. (1986) have suggested that *Mysis* should have increased in Lake Mich-

TABLE 1. Individuals · m^{-2} of *Mysis relicta* in southern Lake Michigan, 1985–89, at 43°N 86° 40′W, 100 m. Areal estimates from oblique tows with Tucker trawl were estimated by multiplying pooled mean concentrations (X_p) from each stratum times the depth interval. Values for replicate samples are listed.

Date (time)	Vertical tows		Oblique tows
	300 μm	130 μm	300 μm
	1985		
11 June (0140)			132
26 June (0110)	98		
18 July (0110)	195		
30 July (2330)	65		
7 Aug. (2310)	48		
21 Aug. (0115)	60		
27 Aug. (0120)	181		
	1986		
4 June (0115)	152	177	
8 July (0130)	42	36	155 145 71
23 July (0045)	958	721	556 346
6 Aug. (0040)	111	111	134 115
26 Aug. (0030)		93 65	
12 Sept. (2130)	23	34 62	
	1987		
30 June (2200)		97	
22 July (2110)		28	
18 Aug. (0100)		33	
2 Sept. (2050)		71	
	1988		
26 May (2145)		149 89 84	
28 June (2240)		111 112 74 68	
19 July (2100)		50 38 52 55	
5 Aug. (0115)		75 94 96 62	
15 Aug. (2230)		36 33 52	
13 Sept. (2310)		57 57 50 59	
	1989		
25 May (2350)		27 23 25	
28 June (0000)		153 113 173	
18 July (2230)		87 121 69	
7 Aug. (2220)		139	
22 Aug. (2230)		61 62 69 87	
27 Aug. (2215)		106 127 139 115	
19 Sept. (2140)		124 112 144	

igan during the past few years. Our results indicate that mysid abundances have not changed appreciably within the past 15 yr. To judge from Beeton's (1960) results, numbers have changed little from values of 30 yr ago.

Evans and Jude (1986) additionally claimed that *Mysis* may be responsible for recent changes in mid-summer abundance and mean size of *Daphnia*, which are vulnerable to *Mysis* (Grossnickle 1982). Our observations show that encounters between *Daphnia* and *Mysis* are minimized because of differences in their vertical distributions at night (Fig. 1). *Mysis* typically avoid the epilimnion, where the *Daphnia* concentrate. Spatial overlap between *Daphnia* and *Mysis* exists for only approximately 5 to 8 h per day during summer, because during the remainder of the day *Mysis* is far below the *Daphnia* population.

We believe that population interactions between *Mysis* and *Daphnia* can be estimated most securely from actual measured vertical distributions of the animals. This attitude was expressed similarly by Murtaugh (1981) who assumed that electivities of *Neomysis* should be assessed with respect to local densities of prey. However, Murtaugh wondered if it were possible for mysids to "take short feeding excursions into the upper waters returning to depth when satiated" (p.896). If such speculation were true, our measured vertical distributions would not be representative of the depth distribution of mortality. Guts dissected from *Mysis* collected below 40 m at ca. 0100 h on 8 July 1986 in fact contained *Daphnia* although the prey were absent in those deep strata (Fig. 1).

Unable to reject Murtaugh's speculation, we decided to evaluate the possibility that *Mysis* could, through brief foraging excursions, obtain *Daphnia* from anywhere in the water column. Consequently, gut contents of specimens in vertical nets towed from 90 m to surface were considered representative for the entire water column. The numbers of *Daphia* consumed by *Mysis* on different dates are summarized in Table 2. Because samples had been collected midway through the night, prey numbers were doubled in order to estimate how many prey might be consumed per mysid per night on different dates. No special consideration was given to *Mysis* body size in this calculation because specimens were drawn randomly from the existing size distribution on each date.

Estimated predation rates from the functional response relationship of Bowers and Vanderploeg (1982) and our in situ estimates of animal abundances are shown in Table 3, as the "Spatial Overlap" Model. In no case did our estimated mortality rates by *Mysis* on Daphnia exceed 1.5% per day, and in eight of 12 cases they were less than 1% per day. When mortality rates were estimated independently from the numbers of prey identified in *Mysis* foreguts, the estimated rates were less than 1% per day in all four cases.

Estimated birth rates for *Daphnia* populations at the offshore reference station during summer range from 4 to 19% · d^{-1} (mean = 10.4%, SD = 4.8%, n = 9; J. T. Lehman, unpubl. data), which are considerably greater than the magnitudes of mortality presumably imposed by *Mysis* (Table 3). We thus regard the hypothesis that *Daphnia* is being controlled by *Mysis* in Lake Michigan to be doubtful. Under other conditions, and in other lakes, mysids may indeed exert profound effects on the population dynamics of *Daphnia*, as results from Lake Tahoe (Goldman et al. 1979; Threlkeld et al. 1980) and Lake Washington (Murtaugh 1981) seem to indicate. We believe that *Mysis* has the potential to change plankton communities when it is released from fish predation. We find no indication, however, that planktivory by fish on *Mysis* has yet diminished in Lake Michigan. Indeed our findings on the decline of *D. pulicaria* (Dorazio et al. 1987) suggest that visual planktivory was accelerating by 1985. The same fish species which Dorazio et al. implicated in planktivory of large *Daphnia (Coregonus hoyi)* switches to a more demersal existence after the first year of its life (Crowder and Crawford 1984), and may thereby exert predation on the benthic mysids.

Warm water refugia for Cladocera have been observed in the Experimental Lakes Area, Ontario (Nero and Sprules 1986), Donner and Fallen Leaf Lakes (Morgan et al. 1981), Pend Orielle Lake (Rieman and Falter 1981), and Kootenay Lake (Zyblut 1970; Daley et al. 1981). Additional evidence that *Daphnia* may evade mysids by seeking warm epilimnetic water at night comes from the in situ predation experiments of Bowers and Vanderploeg (1982). *Mysis* in Lake Michigan feeding at night near the epilimnion metalimnion interface ingested significantly fewer *Daphnia* than did animals placed at the same depths during daytime hours when *Daphnia* are more abundant

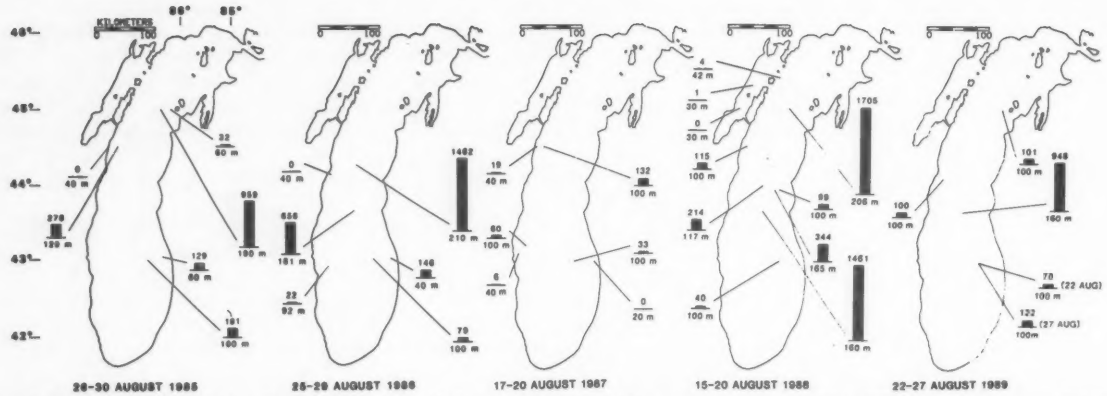

FIG. 2. Areal densities of *Mysis relicta* (individuals · m^{-2}) in Lake Michigan during synoptic cruises, 1985 to 1989. Station depths are listed.

TABLE 2. Consumption of *Daphnia* by *Mysis* in Lake Michigan.

Date	Time	*Mysis* lengths (mm)	*n*	*Daphnia* per gut Mean	SE
26 June 85	0110	8–18	10	0.9	0.4
18 July 85	0110	9–22	9	1.1	0.3
30 July 85	2330	10–18	9	1.6	0.2
6 Aug. 86	0050	7–20	11	3.5	0.8

TABLE 3. Areal abundances of *Daphnia* spp. and *Mysis*, and estimated rates of mortality on *Daphnia* by *Mysis*. *Daphnia* abundances are cruise mean estimates at the offshore reference station, based on diel sampling over 24 h; *Mysis* abundances are means from Table 1. Mortality rates are derived from the vertical distributions shown in Fig. 1, or from consumption rates reported in Table 2.

Date	*Daphnia* (m^{-2})	*Mysis* (m^{-2})	Mortality (% · d^{-1}) Spatial overlap	Prey consumed
		1985		
11 June	1500	132	1.4	
26 June	50500	98	1.12	0.35
18 July	62600	195	1.05	0.69
31 July	105300	65	0.38	0.20
7 Aug.	148800	48	0.50	
21 Aug.	135500	60	0.69	
		1986		
4 June	5900	164	0.12	
8 July	193100	90	0.15	
23 July	44300	645	1.33	
6 Aug.	83200	118	0.05	0.99
26 Aug.	222800	79	0.02	
12 Sept.	67800	40	0.09	

at those depths. We interpret these reported refugia as supporting evidence for our belief that the Spatial Overlap Model is a reliable predictor of population interactions between *Mysis* and *Daphnia*. In summary, evidence indicates that *Mysis* is not now a dominant agent of mortality on *Daphnia* populations in Lake Michigan.

Acknowledgements

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Phylogenetic Relationships Among Members of *Salvelinus* Inferred from Mitochondrial DNA Divergence

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Grewe, P. M., N. Billington, and P. D. N. Hebert. 1990. Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. Can. J. Fish. Aquat. Sci. 47: 984–991.

Mitochondrial DNA (mtDNA) was isolated from five species of the genus *Salvelinus (alpinus, confluentus, fontinalis, malma,* and *namaycush)* and from the closely related taxon, *Hucho hucho*. Restriction sites recognized by 14 endonucleases were mapped for each of the species and percent sequence divergence estimated between species pairs. A phylogeny of *Savelinus* based on restriction analysis of mtDNA, using *Hucho* as an outgroup, readily resolved the three subgenera: *Baione, Cristivomer,* and *Salvelinus*. Within *Baione,* no differences were detected between the mtDNA of brook (*S. fontinalis fontinalis*) and aurora (*S. f. timagamiensis*) trout. However, members of the subgenus *Salvelinus* were more variable such that two groups were resolved, one consisting of *S. alpinus alpinus, S. a. oquassa,* and *S. malma,* and the other of *S. a. stagnalis* and *S. confluentus*. A composite distance phenogram representing the phylogeny of the subfamily Salmoninae was constructed using the sequence divergence data obtained in the current study in conjunction with data from three other salmonid mtDNA studies. Estimates of divergence time between the major taxa of this group suggest that the salmonine genera originated in the late-Miocene to early-Pliocene, while extant species originated during the late-Pliocene to early-Pleistocene.

L'ADN mitochondrial (mtDNA) a été isolé à partir de cinq espèces du genre *Salvelinus (alpinus, confluentus, fontinalis, malma et namaycush)* et du taxon qui y est étroitement relié *Hucho et hucho*. Les sites de restrictions reconnus par 14 endonucléases ont été cartographiés pour chacune des espèces et le pourcentage de divergence de séquence a été estimé entre les d'espèces. Une analyse phylogénique de *Salvelinus* basée sur une analyse des restrictions du mtDNA, *Hucho* étant utilisé comme hors-groupe, a permis de déterminer rapidement les trois sous-genres : *Baione, Cristivomer* et *Salvelinus*. Dans le sous-genre *Baione,* aucune différence n'a été décelée entre le mtDNA de la truite de ruisseau (*S. fontinalis fontinalis*) et celui de l'omble de fontaine (*S. f. timagamiensis*). Toutefois, les membres du sous-genre *Salvelinus* étaient plus variables, si bien que deux groupes ont été déterminés, l'un consistant en *S. alpinus alpinus, S. a. oquassa* et *S. malma* et l'autre en *S. a. stagnalis* et *S. confluentus*. Un phénogramme de la distance composite représentant la phylogénie de la sous-famille des salmonidés a été établi à l'aide des données de divergence de séquence obtenues dans l'étude courante, conjointement aux données provenant de trois autres études de mtDNA chez les salmonidés. Des estimations du temps de divergence entre les principaux taxons de ce groupe laissent croire que le genre salmonîdé date des environs du Miocène supérieur ou du Pliocène inférieur tandis que les espèces ultérieures viennent du Pliocène supérieur au Pléistocène inférieur.

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The Salmonid family includes some 70 species which are subdivided into three different subfamilies: Coregoninae, Thymallinae, and Salmoninae. The subfamily Salmoninae includes six genera: *Brachymystax, Hucho, Oncorhynchus, Salmo, Salmothymus,* and *Salvelinus*. Salmonid taxonomy is currently based largely on morphological studies (e.g. Norden 1961) supplemented by karyological (Simon 1963; Gold et al. 1972), and ontogenetic studies (Balon 1980; Pavlov 1980). More recently, allozyme analysis (Tsuyuki and Roberts 1966; Utter et al. 1973), DNA–DNA hybridization (Mednikov and Akhundov 1975; Hanham and Smith 1980) and chromosome banding studies (Phillips and Ihssen 1985; Phillips et al. 1989) have been used to further resolve the phylogeny of species in this group.

The taxonomy of some salmonine groups, especially members of the genus *Salvelinus*, remains confused (McPhail 1961; Behnke 1980, 1984; Savvaitova 1980). These difficulties have been attributed to the recent origin and hence the limited degree of genetic divergence among the taxa. Most extant genera appear to be of Pliocene or Pleistocene origin (Neave 1958; Norden 1961; Behnke 1980, 1984). Evidence of their recent speciation is demonstrated by the viability of intergeneric, and fertility of intrageneric hybrids (Buss and Wright 1958). Discrimination of taxa at the specific level is further complicated by intraspecific phenotypic plasticity. For example, eggs from a single Arctic char can give rise to three distinct morphotypes (Nordeng 1983). More recent efforts, which have attempted to use allozymes to resolve the taxonomic difficulties encountered in morphological studies, have been complicated by the polyploid nature of the salmonid genome (Allendorf et al. 1979; Clayton and Ihssen 1980; Utter 1981).

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Restriction analysis of mitochondrial DNA (mtDNA) is an alternative approach to studies of the nuclear genome for the assessment of phylogenetic relationships among closely related taxa. Advantages of mtDNA analysis include its maternal inheritance and elevated rate of evolution relative to that of single copy nuclear DNA (Avise et al. 1987; Moritz et al. 1987). It has already proven useful for examining relationships among conspecific individuals of a variety of taxa (Avise et al. 1984, 1987; Billington and Hebert 1988; Grewe and Hebert 1988; Crease et al. 1989). Other studies have utilized mtDNA data sets to infer phylogenetic relationships at higher taxonomic levels in birds (Kessler and Avise 1985), primates (Ferris et al. 1981), and fish (Berg and Ferris 1984; Gyllensten and Wilson 1987; Thomas et al. 1986; Dowling and Brown 1989).

Two techniques are employed to infer phylogeny from restriction site data. One method uses parsimony analysis to represent the phylogeny as a Wagner network and separate the taxa by the numbers of character state changes required (i.e. restriction site changes) to move from one taxon to the next. The other uses estimates of percentage sequence divergence (Nei and Tajima 1983) followed by UPGMA analysis (Sneath and Sokal 1973; Nei et al. 1985) to represent the phylogeny as a bifurcating tree. Using the latter method, an estimation of the approximate time of divergence may be obtained by assuming a constant rate of sequence divergence up to an upper limit of approximately 15% (Brown et al. 1979; Brown 1983). However, these estimates must be interpreted with caution in light of growing evidence for rate variation in mtDNA evolution (Moritz et al. 1987; Bentzen 1988; Dowling and Brown 1989).

This paper attempts to reconstruct a phylogeny, utilizing both sequence divergence estimates and parsimony analysis, for North American members of the three *Salvelinus* subgenera (*Cristivomer, Baione,* and *Salvelinus*), with the closely related taxon *Hucho hucho* employed as an outgroup. In addition, a composite phylogeny for the major genera of the subfamily Salmoninae, based on both the current data and other published salmonid mtDNA data, is presented.

Methods

Samples of all the North American members of the *Salvelinus* genus were collected from nine locations across the continent (Table 1). In addition, six *S. alpinus alpinus* were collected from England; and five *Hucho hucho* were obtained from Czechoslovakia for use as an outgroup. Full details of the origin of the lake trout samples are provided by Grewe and Hebert (1988).

Mitochondrial DNA was extracted from fresh liver tissue and purified using the methods described by Grewe and Hebert (1988) and Billington and Hebert (1988). MtDNA from each fish was restricted with 14 endonucleases having hexanucleotide sequences (*Bam* HI, *Bcl* I, *Bgl* II, *Bst* EII, *Eco* RI, *Hind* III, *Nco* I, *Pst* I, *Pvu* II, *Sal* I, *Sma* I, *Sst* II, *Xba* I, and *Xho* I), using the buffer system and incubation temperatures recommended by the supplier (Bethesda Research Laboratories). The resulting fragments were end-labelled with ^{32}P-labelled nucleotides and separated by electrophoresis in 1.2% agarose and 4% polyacrylamide gels. The gels were dried on filter paper and the fragments visualized by autoradiography (Grewe and Hebert 1988; Billington and Hebert 1988).

Appropriate double digests were used to confirm homologies of restriction sites and to construct restriction site maps for the mitochondrial genome of a single individual of each operational taxonomic unit (OTU). From these data a restriction site presence/absence matrix was created. Mitochondrial genome sizes were estimated by comparing the sums of restriction fragments produced by all 14 endonucleases relative with those of the lake trout. For simplicity, only fish possessing the main lake trout mtDNA clones (A, B, and C — Grewe and Hebert 1988) were used in this study; intraspecific diversity in lake trout mtDNA has been more fully described by Grewe and Hebert (1988).

TABLE 1. Taxa used in this study and their origin.

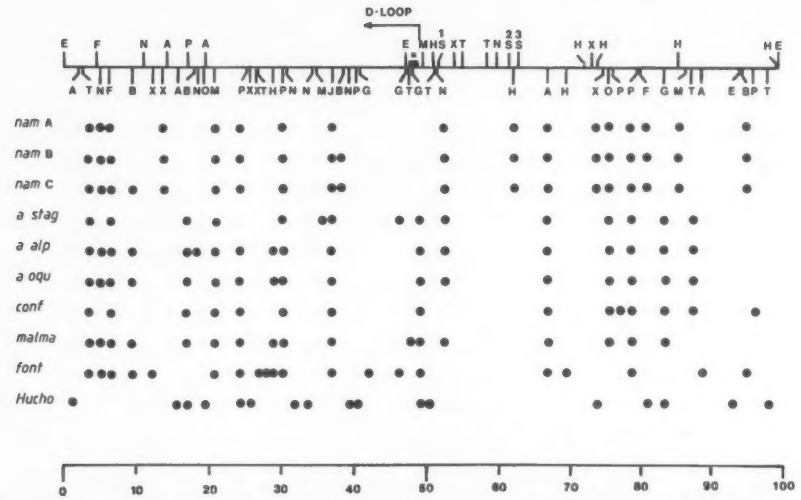
Species	No. of fish	Origin
Hucho hucho (Huchen)	5	Pribovce Hatchery, Bratislava, Czechoslavakia. May 1987
Salvelinus alpinus alpinus (Arctic Charr)	6	Lake Windermere, Cumbria, England. Jan. 1986.
alpinus stagnalis (Arctic Charr)	1	Tarsuk Arm, Baffin Island, N.W.T. Aug. 1985.
	6	Myaktulik, Baffin Island, N.W.T. Aug. 1989.
	9	Iqualulikuluk, Igloolik, N.W.T. Aug. 1989.
alpinus oquassa (Sunapee)	15	Floods Pond, Maine, Oct. 1987.
confluentus (Bull Trout)	2	Peppers Creek, Alberta, Oct. 1987.
fontinalis (Brook Trout)	2	Lake Nipigon, Ontario. July 1986.
	2	Dickson Lake, Ontario. Oct. 1988.
fontinalis timagamiensis (Aurora Trout)	12	Ontario Ministry of Natural Resources, North Bay Fish Culture Unit. Jan. 1988.
malma (Dolly Varden)		Drizzle Lake, Queen Charlotte Is., B.C. June 1987.
namaycush (Lake Trout)	143	Various (see Grewe and Hebert 1988 for details).

The maximum likelihood estimates of the number of nucleotide differences per site (*d*) were calculated following Nei and Tajima (1983) and divergence estimates between taxa are reported as percent sequence divergence. These data were used to construct a distance phenogram by UPGMA (Sneath and Sokal 1973), with the standard error values of the branching points calculated by the formulae of Nei et al. (1985).

A parsimony network or "Wagner Tree" was constructed using Swofford's (1985) PAUP program, with *Hucho hucho* as an outgroup to root the network. Initially the program was run with all 10 OTUs, then the branch and bound search routine of Hendy and Penny (1982) was used to determine the most parsimonious trees. In addition, the tree topology obtained by UPGMA was compared with that of the Wagner network using the TOPOLOGY option.

Results

Ten mitochondrial clones were found among the nine taxa examined. Three of these clones were identified in lake trout (*Salvelinus namaycush*) and have been previously described (Grewe and Hebert 1988). No variation was detected among members of the other taxa, with the exception of *S. alpinus alpinus*, where two of the six fish showed a single *Nco* I site loss. However, as these two individuals possessed an identical restriction fragment pattern to *S. a. oquassa*, they were treated as the same mtDNA clone in later analyses. It is worth noting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E F N A P A E MHS XT TNSS H X H H HE
A TNF B XX ABNOM PXXTHPN N MJBNPG GTGT N H A H XOPP F GMTA E BP T
nam A
nam B
nam C
a stag
a alp
a oqu
conf
malma
font
Hucho
0 10 20 30 40 50 60 70 80 90 100

FIG. 1. Restriction site maps of mtDNA from *Hucho hucho* and nine *Salvelinus* taxa. Endonucleases: A = *Bcl* I, B = *Bam* HI, E = *Eco* RI, F = *Pst* I, G = *Bgl* II, H = *Hind* III, J = *Sal* I, M = *Sma* I, N = *Nco* I, O = *Xho* I, P = *Pvu* II, S = *Sst* II, T = *Bst* EII, X = *Xba* I. The molecules have been depicted linearly, opened at a conserved *Eco* RI site. Invariant sites are shown above the line, while variable sites are shown below the line with filled circles representing their presence in a particular taxon. A map scale of 0–100 arbitary units is included. The location of a length insert in *Hucho hucho* is shown by an asterisk.

TABLE 2. Matrix of percent sequence divergences (above diagonal) and numbers of shared restriction sites (below diagonal) among mitochondrial types for nine *Salvelinus* taxa and *Hucho hucho*. A, B, and C refer to the three *S. namaycush* clones. Names of other *Salvelinus* taxa are abbreviated. In the case of *S. alpinus*, the abbreviation refers to the subspecific name.

	A	B	C	*stag*	*alp*	*oqu*	*conf*	*malma*	*font*	*Hucho*
A	38	0.22	0.43	3.50	3.08	2.86	3.50	2.86	4.04	7.66
B	38	39	0.21	3.72	3.30	3.08	3.72	3.08	4.25	7.88
C	38	39	40	3.94	3.00	2.78	3.94	2.78	3.93	8.09
stag	30	30	30	36	1.63	1.41	1.45	1.91	4.16	7.20
alp	32	32	33	34	39	0.22	1.63	0.44	3.21	6.98
oqu	32	32	33	34	38	38	1.41	0.45	3.00	6.98
conf	30	30	30	33	34	34	36	1.91	4.16	6.53
malma	32	32	33	33	37	37	33	38	3.00	6.98
font	31	31	32	30	33	33	30	33	41	9.01
Hucho	24	24	24	24	24	25	25	25	23	38

that the brook char samples representing two sub-species (*S. fontinalis fontinalis* and *S. f. timagamiensis*) had identical mtDNA restriction fragment patterns.

Sixty-eight restriction sites were mapped among the 10 mtDNA clones (Fig. 1). From these maps the number of shared and unique restriction sites among the mtDNA clones were determined (Table 2); sites falling within 0.5 map units (approximately 80 base pairs (bp)) of each other were considered homologous. A total of 21 different restriction sites were shared by all taxa, while 28 sites were conserved among *Salvelinus* congeners. On average, *Hucho hucho* shared 24 sites with the *Salvelinus* species. Single site gains/losses were required to move between patterns of the *Salvelinus* mtDNA clones, while multiple site gains/losses were required to move from any of the *Salvelinus* patterns to those observed in *Hucho hucho*.

Double digests employing combinations of the enzymes *Eco RI*, *Sal* I, and *Sst* II produced fragments of equal mobility in all *Salvelinus* taxa examined. Thus, the mitochondrial genomes of these species, by comparison of their digestion profiles relative to those of the lake trout, were estimated to be approximately 16 800 ± 200 bp in length. However, the mitochondrial genome of *Hucho hucho* was approximately 700 bp longer than the *Salvelinus* genome. This length increase was the result of a single insertion which mapped to the area between the *Eco* RI (E) and *Sma* I (M) restriction sites at 48 and 51 map units, respectively (Fig. 1).

Sequence divergence estimates (Table 2) within taxa varied from 0.21–0.43% among *S. namaycush* clones, to 0.22–1.63%, among subspecies of *S. alpinus*. Differences among taxa varied considerably, ranging from 0.44–1.91% between *S. malma* and *S. alpinus* taxa, 2.7–4.25% among *Salvelinus* subgenera and 6.53–9.01% between *Salvelinus* and *Hucho*.

The UPGMA phenogram generated from these data (Fig. 2) showed that *Salvelinus* was clearly resolved from *Hucho* and that members of the three subgenera of *Salvelinus* were also distinct. Thus, one group consisted of the lake trout clones, another of the brook trout, while the third group consisted of all the *S. alpinus* sub-species, along with *S. malma* and *S. confluentus*. This third group was further divided into two subgroups, one including Arctic char from northern Canada (*S. a. stagnalis*) and bull trout (*S. confluentus*), while the other included Dolly Varden (*S. malma*), Sunapee (*S. a. oquassa*), and Arctic char from Britain (*S. a. alpinus*).

When cladistic analysis was carried out using the Branch and Bound option of PAUP, six equally parsimonious trees (tree length = 56; consistency index = 0.839) were found. These trees were identical in overall structure, the differences occuring in the relative ordering of the lake trout clones and of the *malma*, *a. alpinus*, and *a. oquassa* branches. These differences are the

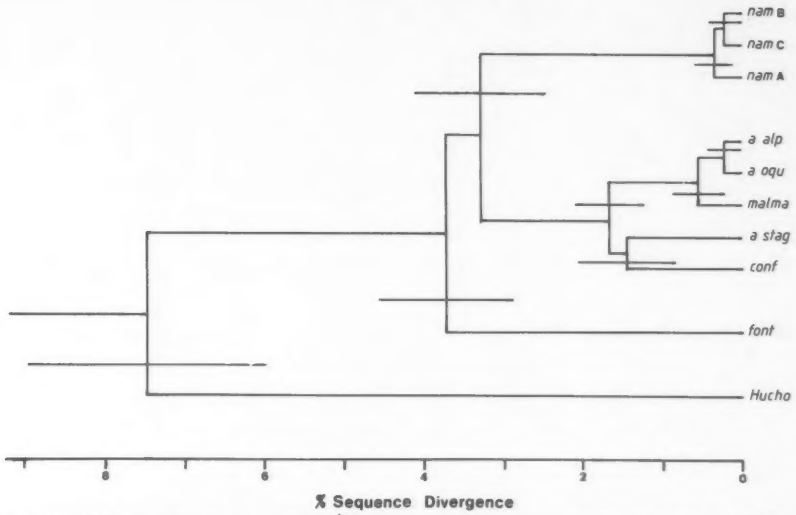

FIG. 2. UPGMA phenogram of percent sequence divergence among nine *Salvelinus* taxa and *Hucho hucho*, generated from mtDNA restriction site data, with branch points ±1 SE.

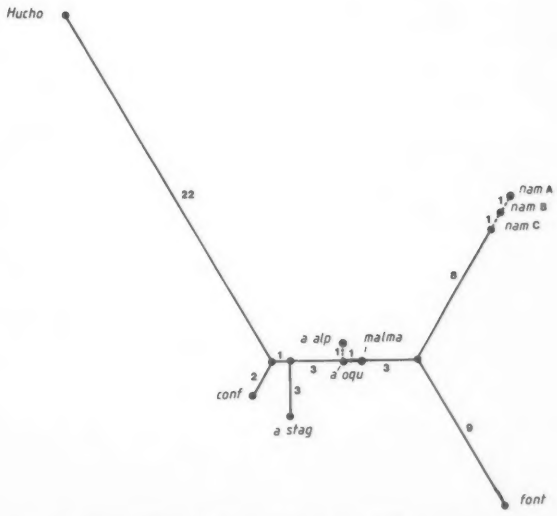

FIG. 3. Wagner network for seven *Salvelinus* taxa using *Hucho* as an outgroup (solid lines); tree length = 53, consistency index = 0.868. Branches are drawn in proportion to their lengths and the number of character state changes is shown on each branch. The positions of two lake trout clones (*nam* A and B) and Arctic char (*a. alp*) were added after analysis (dotted lines) as they differed only in autapomorphies.

result of autapomorphies, that is, characters in which one state is found in a single taxon and another alternative state is found in all other taxa (Swofford 1985). Such characters are phylogenetically uninformative. Therefore, the lake trout A and B clones, and *S. a. alpinus* were excluded from analysis and PAUP was re-run. Only one Wagner network (Fig. 3) was obtained for the seven remaining taxa (length = 53; consistency index = 0.868), even after analysis by the branch and bound procedure. Lake trout clones A and B and *S. a. alpinus* were then superimposed onto this network. Finally, a UPGMA topology obtained by re-analysis of the reduced data set was input into PAUP and examined using the TOPOLOGY procedure. This produced a less parsimonious network (tree length = 56; consistency index = 0.821).

Discussion

Mitochondrial genome structure

The present study has provided a substantial amount of information concerning the nature of mtDNA variation in the Salmonidae. The length of the mitochondrial genome in all members of the genus *Salvelinus* examined was found to be approximately 16 800 ± 200 bp. This value is similar to length estimates available for the mtDNA molecules of other fish species (Billington and Hebert 1988), including salmonids (Berg and Ferris 1984; Birt et al. 1986; Thomas et al. 1986). However, the *Hucho hucho* mitochondrial genome was approximately 700 bp longer. Such mtDNA size increases are not unusual as rare variants within single taxa. For example, length insertions of approximately 300 bp and 1700 bp have been observed in walleye (*Stizostedion vitreum*) mtDNA (Billington and Hebert 1988; Ward et al. 1989), while Bermingham et al. (1986) report that the mtDNA of bowfin (*Amia calva*) varied by as much as 900 bp overall, and by 700 bp among fish from a single river system. However, it appears from the present analysis that the length increase is fixed in *H. hucho*. It would be of interest to ascertain the presence or absence of a similar size shift in other *Hucho* species. As noted in other vertebrates (Moritz et al. 1987), the length insert in *Hucho* mapped to the boundary of the control (D-loop) region (see below).

There is now enough information on the mitochondrial genomes of animal taxa to make it clear that gene order is highly conserved. For example, sequencing studies have shown that gene order is identical in four different vertebrates (*Homo* — Anderson et al. 1981, *Mus* — Bibb et al. 1981, *Bos* — Anderson et al. 1982, and *Xenopus* — Roe et al. 1985). Joint

mapping/hybridization studies have confirmed that a similar gene order exists in other vertebrates (Moritz et al. 1987) including fish (*Cyprinus carpio* — Araya et al. 1984, *Salmo salar* — Davidson et al. 1989, and six *Oncorhynchus* species — Thomas and Beckenbach 1989). The conservation of gene order means that the location of specific segments of the genome, such as the D-loop region, can be identified if three fixed sites are found to serve as a reference for both location and polarization of position on the circular mitochondrial genome. There appears to have been little effort made to locate such sites in the past, although it has been noted that some restriction sites are conserved in many, if not all vertebrates. For example, two *Sst* II (CCGCGG) sites are present approximately 1.7 kilobase pairs (kbp) apart in the 12S and 16S ribosomal RNA (rRNA) genes of four mammals (*Homo, Bos, Mus, Rattus*) and one amphibian (*Xenopus*), as well as a number of other vertebrates (H. Hotz, C. Spolsky, and T. Uzzell, Department of Ecology, Ethology, and Evolution, University of Illinois, Urbana, IL, USA, pers. comm.).

In this study, three conserved *Sst* II sites were found in the mitochondrial genomes of *Salvelinus* and *Hucho*, occurring at map positions 51 (S1), 61 (S2), and 63 (S3), representing fragments of 1.75 and 0.25 kbp (Fig. 1). Berg and Ferris (1984) similarly observed a 1.75 kbp *Sst* II fragment in four salmonids, but failed to detect the smaller fragment, because their limit of resolution was 0.5 kbp. These three sites appear to occur widely in fish as they were present in five percid species (Billington and Hebert 1988; Billington et al. 1988; N. Billington, unpubl. data). The length (1750 bp) of the larger fragment in fish appears to be more consistent with its length in *Xenopus* (1727 bp), than in mammals (1688 bp in *Mus*, 1686 in *Bos*, and 1669 bp in *Homo*).

The third *Sst* II site (S3, Fig. 1), located 250 bp downstream of S2, was conserved in all the salmonids and percids examined, but is not present in mammalian or amphibian sequences. However, a *Sst* II one-off site (CCTCGG) occurs in the 16S rRNA gene of *Bos, Homo*, and *Mus* mtDNA sequences, approximately 250 bp away from the *Sst* II site in the 16S rRNA gene. Furthermore, a similarly located *Sst* II semi-site (CCACGG) occurs 250 bp from the highly conserved 16S rRNA *Sst* II site in the *Xenopus* mtDNA sequence (Roe et al. 1985). By assuming that this extra *Sst* II site occurs in the 16S rRNA gene, we were able to align the 1750 and 250 bp *Sst* II fragments observed in *Salvelinus* and *Hucho* mtDNA with the *Bos, Homo, Mus*, and *Xenopus* genomes (Fig. 4). Following such orientation, it was then possible to determine the approximate position of the D-loop. The D-loop regions in *Mus, Bos*, and *Homo* begin approximately 500–700 bp upstream from the 12S rRNA *Sst* II site (S1) and span approximately 1000 bp. Thus, the D-loop in *Salvelinus* and *Hucho* mtDNA is tentatively located between map units 42 and 49 (Fig. 1), with the direction of heavy strand replication being from left to right.

Salvelinus taxonomy

Prior to considering a phylogeny inferred from mtDNA analysis, it is necessary to review the present state of *Salvelinus* taxonomy. Classical taxonomy (McPhail 1961; Behnke 1980, 1984) now recognizes the lake trout, brook trout, and Arctic char as the type representatives of the three subgenera of *Cristivomer, Baione*, and *Salvelinus*, respectively. The subgenera *Cristivomer* and *Baione* are both endemic to North America and each includes a single species, with two extant subspecies (*S. namaycush namaycush* and *S. n. siscowet; S. fontinalis fontinalis* and *S. f. timagamiensis*). The subgenus *Salvelinus* has an Holarctic distribution and includes at least five species. Four of these species are monotypic (*S. albus, S. confluentus, S. leucomaenis*, and *S. malma*) and are endemic to the North American and Asian coastal regions of the north Pacific. However, *S. alpinus* includes at least five subspecies and has an Holarctic distribution. *Salvelinus alpinus alpinus* is common in northern Europe, including Britain and Iceland, while *S. a. stagnalis* is distributed across northern Canada. A third subspecies, *S. a. oquassa*, is restricted to Maine, USA. The two remaining subspecies comprise a group found in alpine lakes of central Europe (*S. a. salvelinus*) and a Siberian group (*S. a. erythrinus*).

The genus *Hucho* includes five species, which are broadly distributed across Eurasia (Holcik 1982; Holcik et al. 1988). Only the Danubian huchen, *Hucho hucho hucho*, was examined in the present study. Formerly, members of this genus were placed in either *Salmo* or *Salvelinus*. However, most authors now agree upon the independent generic status of *Hucho* and consider it to be most closely related to *Salvelinus* and *Branchymystax* (Holcik 1982; Holcik et al. 1988).

Salvelinus mtDNA variation

Both the phenetic and cladistic analyses discriminated the three *Salvelinus* subgenera, but it was not possible to determine

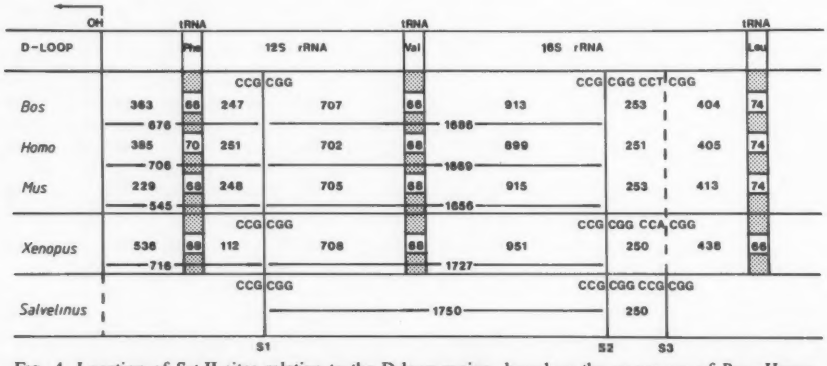

FIG. 4. Location of *Sst* II sites relative to the D-loop region, based on the sequences of *Bos, Homo, Mus*, and *Xenopus* mtDNA (see text for details). Lengths between sites and genes are in base pairs. OH = origin of heavy strand replication.

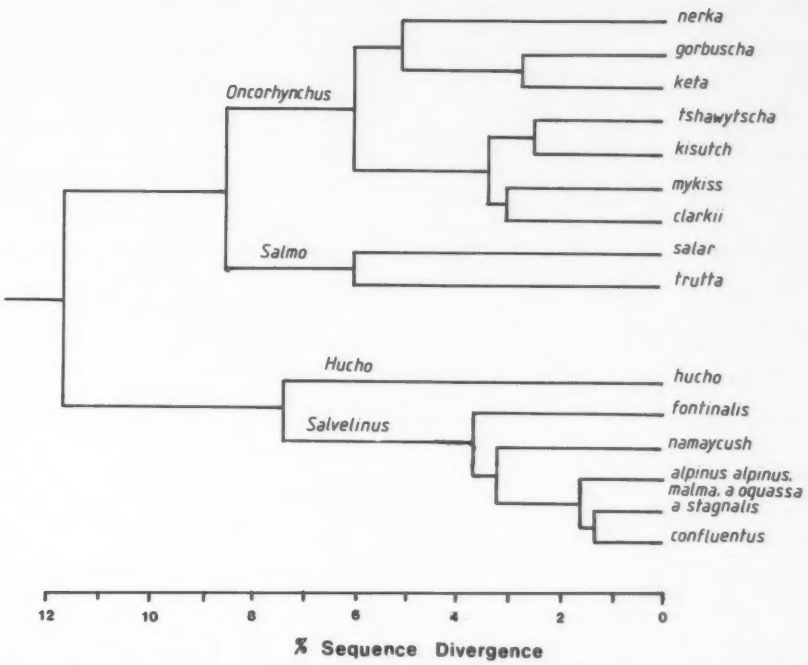

FIG. 5. Composite distance phenogram of the Salmoninae showing percent sequence divergence of mtDNA based on current data and published values of Berg and Ferris (1984); Thomas et al. (1986); and Gyllensten and Wilson (1987).

their exact branching order. Thus, at present these three groups represent an unresolved trichotomy. In fact, different results were obtained with the phenetic and cladistic analyses. The parsimony network placed the *Salvelinus* subgenus closest to the outgroup *Hucho* (Fig. 3), while the phenetic analysis suggested that this subgenus was the most divergent (Fig. 2). However, the topology obtained from the UPGMA phenogram was found to be less parsimonious when compared with the Wagner network.

Within the subgenus *Baione*, there were no differences between brook (*S. f. fontinalis*) and aurora (*S. f. timagamiensis*) trout mtDNA, providing further support for the arguments against retaining the subspecific status of the aurora trout (see Parker and Brousseau 1988). Within the subgenus *Cristivomer*, mtDNA variation in *S. n. namaycush* has been previously documented by Grewe and Hebert (1988), and the present study did not involve analysis of the other subspecies (*S. n. siscowet*).

Classical taxonomical studies on the subgenus *Salvelinus* (Behnke 1980, 1984) have recognized two sub-groups: an Arctic char group consisting of the *S. alpinus* sub-spcecies and a Dolly Varden group consisting of *S. malma* and *S. confluentus*. In fact, the latter species is often assumed to be the land-locked form of *S. malma*. In contrast, mtDNA analysis suggested that *S. confluentus* is most closely related to *S. a. stagnalis*, while *S. malma* is more closely related to *S. a. alpinus* and *S. a. oquassa*. The mtDNA results are consistent with data on chromosomal variation, which show that *S. malma* and *S. alpinus* are more closely related to each other than either is to *S. confluentus* (Cavender 1984; Phillips et al. 1989), but more research is required on the taxonomy of this subgenus.

Mitochondrial DNA sequence divergence has been estimated at 2% per million yr in several mammalian lineages and has been estimated to remain linear for approximately 8 million yr (Brown et al. 1979; Brown 1983). Other studies on vertebrates have generally corroborated this value (Wilson et al. 1985, and references therein; Shields and Wilson 1987). Assuming the same rate of differentiation, it is possible to calculate the approximate divergence times of the various salmonid mtDNAs examined in the present study. The three mtDNA clones present in *S. namaycush* show less than 0.5% sequence divergence and appear to have differentiated since the mid-Pleistocene (100 000–250 000 ybp). By comparison, the two groups within the subgenus *Salvelinus* were more divergent (1.65%), suggesting they last shared a common ancestor approximately 800 000 ybp (early Pleistocene). Similarly, the three subgenera of *Salvelinus* last shared a common ancestor approximately 1.55–1.85 million ybp, when they simultaneously diverged from each other. This value agrees with a late-Pliocene/early-Pleistocene separation of these groups from a common ancestral *Salvelinus* as hypothesized by Behnke (1980). The mtDNA of *H. hucho* was clearly differentiated (7.5% sequence divergence) from that of *Salvelinus*, suggesting that these two genera diverged from a proto-*Hucho*/*Salvelinus* ancestor approximately 4 million ybp (mid-to late-Pliocene).

In a broader taxonomic sense, it is possible to create a composite phylogeny (Fig. 5) for the genera of the subfamily Salmonidae, excluding *Brachymstax* and *Salmothymus* (which have not been evaluated), by combining the percent sequence divergence data obtained in the current study with those of prior mtDNA studies (Berg and Ferris 1984; Thomas et al. 1986;

Gyllensten and Wilson 1987). Such analyses indicate that two main divisions occur within the Salmonidae, with approximately 10–12% sequence divergence between the *Salvelinus*/*Hucho* and the *Salmo*/*Oncorhynchus* lineages. Thus, *Salvelinus* appears to be an old lineage, diverging from the rest of the Salmonidae approximaely 5–6 million ybp, while *Hucho* diverged from the *Salvelinus* lineage just after this break point, approximately 3–4 million ybp.

Estimates of the "true" divergence times among the major salmonine groups are difficult to obtain due to the paucity of fossil remains. Thus, it was not possible to calibrate a salmonid mtDNA clock. However, using the mammalian mtDNA clock once again, it would appear that all modern-day Salmonidae evolved within the last 6 million yr, based on the 12% percent sequence divergence since they last shared a common ancestor. This figure is more recent than that suggested by the limited fossil evaluations. For example, Behnke (1980) suggested that the Salmonines diverged from the ancestral Salmonidae by the Miocene (12–25 million ybp), while Cavender (1980) has identified a 10-million-year old fossil of *Salvelinus* from Nevada.

The disagreement between the fossil data and mtDNA data is unlikely to be due to homoplasy affecting the sequence divergence values, which are less than 15% and thus, well within the linear portion of the sequence divergence/divergence time curve (Brown et al. 1979; Brown 1983). Therefore, these values are not likely to be underestimates of the amount of sequence divergence among the taxa. However, it can be argued that mtDNA divergence may have occurred at a rate of less than 2% per million years in the Salmonidae. In fact, Bentzen (1988) recently suggested that, based on fossil records, rates of mtDNA divergence in the Clupeid genus *Alosa* may be approximately 1/10 of those in mammals.

Before dismissing the constancy of mtDNA sequence divergence, it is important to recognize that inadequacies in the fossil record may be a source of apparent error in calibrating the rate of mtDNA evolution. For example, Kessler and Avise (1985) suggested that the apparent deceleration in the rates of avian mtDNA and protein evolution when compared with the paleontological data, might be attributable to the poor state of the fossil record. Moreover, Kessler and Avise (1985) reviewed the controversy concerning the calibration of primate evolution based on fossil and molecular data. Initially, these data were incompatible, with the fossil data favouring a more ancient divergence of humans from African apes. However, in the light of the molecular data, a reevaluation of the fossil data led many paleontologists to view more favourably the possibility of a more recent human–chimp split. The results of the current study suggest the value of a similar critical examination of the salmonid fossil record.

In summary, the present study confirms that the genus *Salvelinus* is a monophyletic group most closely related to the Salmonine genus *Hucho*. Furthermore, three sub-groups within *Salvelinus* were resolved which correspond to the three recognized subgenera. However, the mtDNA data suggest that further taxonomic research needs to be conducted within the subgenus *Salvelinus*. Moreover, the mtDNA data suggests that the Salmonidae can be divided into two major groups which last shared a common ancestor some 6 million yr ago. More detailed analysis of both subspecies and remaining species in the genus *Salvelinus* should further resolve the phylogeny of this group.

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Recent Trends in the Abundance of Harbour Seals, *Phoca vitulina*, in British Columbia

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Aerial censuses of harbour seals (*Phoca vitulina*) were conducted in the Strait of Georgia (1966–88), the lower Skeena River (1977–87), off the southwest coast of Vancouver Island (1976–87), off the northeast coast of the Queen Charlotte Islands (1986), in Jervis Inlet (1987), and at the entrance to Queen Charlotte Strait (1988). The estimated number of seals in the Strait of Georgia, the primary study area, increased from 2170 in 1973 to 15 810 in 1988; the number in the lower Skeena River from 520 in 1977 to 1590 in 1987; and the number off southwestern Vancouver Island from 210 in 1976 to 1130 in 1987. The trends indicated that populations throughout British Columbia had been increasing at a rate of about 12.5% $\cdot$ yr^{-1} since 1973. Based on the density of seals in the areas surveyed, and the relative distribution of bounty and commercial kills, the total post-pupping population in British Columbia was estimated to have numbered 75 000–88 000 in 1988, compared with 9000–10 500 when the species was protected in 1970. Despite the recent increases, which probably reflect the recovery from historic kills, there was no evidence of density-dependent changes in the population growth rate.

Des dénombrements aériens des phoques communs (*Phoca vitulina*) ont été effectués dans le détroit de Géorgie (1966–88), le cours inférieur de la rivière Skeena (1977–87), au large de la côte sud-ouest de l'Île-de-Vancouver (1976–87), au large de la côte nord-est des îles de Reine-Charlotte (1986), dans l'Inlet Jervis (1987), et à l'entrée du détroit Reine-Charlotte (1988). Le nombre estimatif de phoques dans le détroit de Géorgie, la principale région d'étude, est passé de 2 170, en 1973, à 15 810, en 1988; leur nombre dans le cours inférieur de la rivière Skeena est passé de 520 en 1977 à 1590 en 1987; au large du sud-ouest de l'Île-de-Vancouver, de 210 en 1976 à 1130 en 1987. Les tendances indiquaient que les populations de la Colombie-Britannique augmentaient à un rythme d'environ 12.5 %$\cdot$an^{-1} depuis 1973. D'après la densité des phoques dans les régions recensées et la distribution relative des abattages pour des primes et des abattages commerciaux, la population totale après mise bas en Colombie-Britannique a été établie à entre 75 000 et 88 000 animaux en 1988, comparativement à 9000 à 10 500 lorsque l'espèce était protégée en 1970. Malgré les augmentations récentes, qui reflètent probablement la récupération depuis les abattages historiques, on n'a relevé aucune données à l'appui de changements dépendants de la densité dans le rythme de croissance de la population.

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The Pacific harbour seal, *Phoca vitulina richardsi*, inhabits inshore waters from central Baja California northward to Bristol Bay, Alaska, and westward to the Aleutian and Pribilof Islands (Shaughnessy and Fay 1977; Bigg 1981). The species is common in coastal areas, inlets, and estuaries throughout British Columbia and occurs in some rivers and lakes (Fisher 1952; Spalding 1964; Bigg 1969a). Although local movements associated with feeding, breeding, and moulting have been reported (Fisher 1952; van Bemmel 1956; Vaughn 1971; Paulbitski and Maguire 1972; Brown and Mate 1983; Jefferies 1986; Thompson 1989), the species is considered to be non-migratory (Bigg 1981).

Harbour seals are considered a nuisance by many fishermen because they sometimes remove fish from fishing gear and are known to prey on valuable fish species (Imler and Sarber 1947; Fisher 1952; Spalding 1964; Mate 1980; Beach et al. 1985). Earlier in this century, control programs were undertaken to reduce populations throughout much of the northeastern Pacific, including southeastern Alaska (Johnson and Jefferies 1977; Pitcher 1977), British Columbia (Fisher 1952; Bigg 1969a), Washington State (Scheffer and Slipp 1944; Newby 1973), Oregon (Pearson and Verts 1970) and, to a lesser extent, California (Bonnot 1928; Harvey 1987; Stewart et al. 1988). Substantial numbers were also harvested for pelts in British Columbia and Alaska during the 1960s (Bigg 1969a; Johnson and Jefferies 1977). During 1913–69, an estimated total of 200–240 000 seals were killed in British Columbia (P. F. Olesiuk and M. A. Bigg, unpubl. data). However, the species has been protected in British Columbia since 1970 and in the United States since 1972.

Censuses conducted in Washington State, Oregon, and California suggest that harbour seals have been increasing in abundance in recent years (Calambokidis et al. 1985; Brown 1986; Jefferies 1986; Harvey 1987; Boveng 1988). However, previous estimates of the abundance of harbour seals in British Columbia have been too crude and/or subjective to assess population trends. Based on observations made on field trips during 1958–63, Spalding (1964) subjectively estimated there was about one seal per mile of shoreline, or 17 000 seals province-wide. He also cited an estimate of 20 000 similarly made by two sealers who had hunted throughout British Columbia. Using life tables, Bigg (1969a) calculated that the population must have averaged

at least 35 000 during 1913–64 to have sustained the number of bounty kills taken during this period.

During 1966–88, we undertook aerial census of harbour seals in several regions of British Columbia. Since 1973, the timing of censuses, census techniques, and tidal and weather conditions during censuses were standardized. In this report, we analyze these data to establish recent trends in abundance. We also estimate the likely proportion counted during censuses and derive improved estimates of the total population in British Columbia based on the density of seals observed in the surveyed areas and on the relative distribution of bounty and commercial kills.

Methods

Study Area and Duration

Aerial harbour seal censuses (71 flights) were conducted in six areas of British Columbia: the Strait of Georgia, the north and east coasts of the Queen Charlotte Islands, the southwest coast of Vancouver Island, the lower Skeena River, Jervis Inlet, and in the entrance to Queen Charlotte Strait (Fig. 1). The Strait of Georgia was partitioned into seven subareas (SGULF, BBAY, FRASERR, HOWESD, GULFISL, NEGULF, and NWGULF), the Queen Charlotte Islands into three subareas (MASSET, NEQCI, and SEQCI) and the southwest coast of Vancouver Island into two subareas (SWVANISL and BARKLYSD) (see Fig. 1). The subarea boundaries were delineated in the mid-1980s based on areas that had been previously surveyed as a unit (for consistency) and which could be conveniently surveyed during a single flight. Exceptions were BBAY, FRASERR, and HOWESD, which could be surveyed together on one flight.

The primary study area was the Strait of Georgia (56 flights). Traditional haulouts in this area were first noted in the mid-1960s by M. A. Bigg (unpubl. data) during field studies and conversations with seal hunters. Subareas BBAY, FRASERR, GULFISL, and a portion of SGULF were first surveyed in 1966. All subareas were surveyed at least once and most were surveyed twice during 1973–74 with the exception of the NWGULF, which was first surveyed in 1976. Since 1982, three to seven ($\bar{X} = 4.3$) of the subareas were surveyed annually. Duplicate censuses were conducted in BBAY–FRASERR in 1985 and 1986 and in the SGULF in 1987. In 1988, the entire Strait of Georgia was surveyed once just prior to and once toward the end of the pupping season (14 flights), and a portion was surveyed a third time during the autumn moult (four flights).

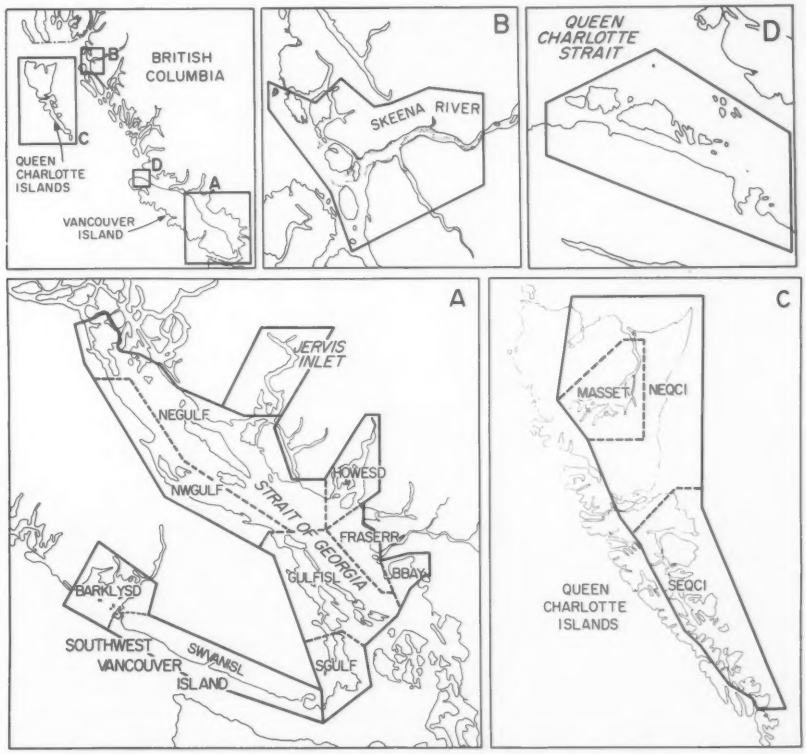

FIG. 1. Study areas in British Columbia: (A) the Strait of Georgia, southwest coast Vancouver island, and Jervis Inlet; (B) the lower Skeena River; (C) the Queen Charlotte Islands; and (D) the entrance to Queen Charlotte Strait. Solid lines delineate areas and dashed lines delineate subareas. Subarea codes: SGULF = southern Strait of Georgia; BBAY = Boundary Bay; FRASERR = Fraser River estuary; HOWESD = Howe Sound; GULFISL = Gulf Islands; NEGULF = northeast Strait of Georgia; NWGULF = northwest Strait of Georgia; SWVANISL = southwest Vancouver Island; BARKLYSD = Barkley Sound; MASSET = Masset Inlet; NEQCI = northeast Queen Charlotte Islands; and SEQCI = southeast Queen Charlotte Islands.

Censuses in the lower Skeena River and off the southwest coast of Vanouver Island provided additional information on trends in abundance. Traditional haulouts in the lower Skeena were first described by Fisher (1947, 1952). Duplicate aerial censuses were conducted in the lower Skeena River in 1977, 1983, and 1987 (six flights). The southwest coast of Vancouver Island was surveyed first in 1976 and again in 1987 (four flights).

In addition, the north and east coasts of the Queen Charlotte Islands were surveyed in 1986 (three flights), Jervis Inlet in 1987 (one flight), and the entrance to Queen Charlotte Strait in 1988 (one flight). Although trends in abundance could not be assessed from these censuses, they provided information on the density of seals in other regions and thus facilitated improved estimates of the total number of seals in British Columbia.

Census Techniques

Aerial censuses were conducted from small, fixed high-wing aircraft, typically a Cessna 172 or 185 or de Havilland Beaver, flown at an altitude of about 125 m and an airspeed of 125 km · h^{-1}. Shorelines were followed and all islands circumnavigated at a distance of about 100 m. All known haulout sites were checked and one to three observers scanned, usually with the aid of 8 × 40 binoculars, for other haulouts, and swimming animals. Visual counts were made of swimming animals and small groups (<5–10) of hauled out animals. Larger groups were usually photographed with a hand held 35-mm SLR camera equipped with a motor drive and 135–200-mm lens using high speed Ektachrome (ISO 400–800) or Kodachrome (ISO 200) colour slide film and subsequently counted from projected transparencies.

Censuses since 1973 were conducted under standardized conditions during which maximum numbers of seals were hauled out (see Results). Most importantly, censuses were timed to coincide with low tides that occurred between approximately 08:00–11:30 PDT. Summer tides in British Columbia are typically semi-diurnal, though successive daily lows often vary considerably in height (Canadian Tide and Current Tables; Canadian Hydrographic Service). Censuses began 2–2.5 h prior to the lower daily low tide and ended just before or just after low tide. The precise point at which counts were initiated and terminated was dictated largely by observations of seals made during the census (see Results). Censuses were conducted on weekdays so as to minimize disturbance by recreational boaters and not during inclement weather (e.g. rough seas, precipitation). During such conditions, it appeared seals were less inclined to haul out and seals in the water were more difficult to count. Censuses were generally conducted toward the end of the pupping season (see next section). These preconditions limited censuses in a given region and year to usually two tidal cycles, each lasting 3–4 d.

Count Adjustments

Prior to analysis of population trends, adjustments were made to counts to account for seals on haulout sites that may not have been surveyed during census, M_{it}, and for seasonal differences in the timing of census, B_{it},

$$(1)\quad C_{it} = C'_{it} \cdot (1 - M_{it})^{-1} \cdot B_{it},$$

where C'_{it} denotes the actual count for the ith subarea in the tth year and C_{it} the corresponding adjusted count. Because flight paths varied slightly between years, a few haulout sites within a subarea, particularly those near its periphery, were sometimes not surveyed. The proportion of the total number of seals in the subarea on missed sites, M_{it}, was estimated from the proportion of the total subarea count on the missed sites in the closest preceding or proceeding complete census of the subarea. With one exception (SGULF in 1966), the corrections were minor as coverage ranged from 91.5 to 100% ($\bar{X} = 99.1\%$).

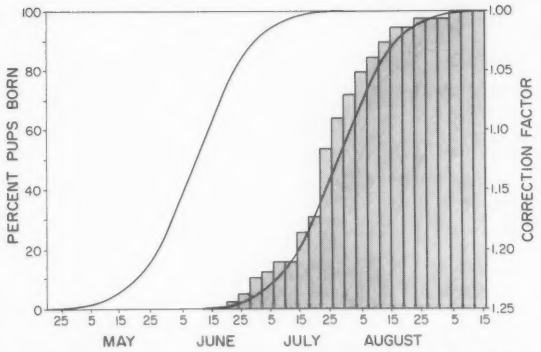

FIG. 2. Pupping curves for the Strait of Georgia and southern British Columbia (right) and for the Skeena River and northern British Columbia (left). The shaded histogram indicates the cumulative number of neonates observed in 4-d intervals in the Strait of Georgia (from Bigg 1969a).

The second correction factor, B_{it}, accounted for differences in the dates of censuses, which was critical when censuses were conducted at different stages of the pupping season. Life tables for harbour seals in British Columbia indicated that pups comprised 20.4% of the total (including pups) post-pupping population (Bigg 1969a). This figure was similar to the composition of pups reported at the end of the pupping season in other areas: 18.6% in the Shetland Islands (Venables and Venables 1955), 19.9–23.8% in Atlantic Canada (Boulva and Maclaren 1979), 20.8% in Ireland (Summers et al. 1980), 16.3–21.4% and 14.2–17.8% in Netarts and Tillamook Bays, Oregon (Brown and Mate 1983), and 16.7% in Puget Sound (Calambokodis et al. 1985). Thus, if we assume that pups constituted 20% of the post-pupping population, the population would increase by 25% during the brief pupping season. As a result, a series of counts conducted progressively later in the pupping season would tend to exaggerate the true rate of increase and vice versa (Jefferies 1986).

The potential for this bias was minimized by adjusting all counts to post-pupping levels. Bigg's (1969a) observations of neonates in the Strait of Georgia indicated that pupping was normally distributed over time (Shapiro and Wilke's (1965) small sample procedure; $W = 0.976$, $n = 39$, $P = 0.648$) with a mean pupping date, μ, of 208 (27 July) and standard deviation, σ, of 16.1 d (Fig. 2). Accordingly, correction factors to account for births subsequent to the Strait of Georgia censuses, B_{it}, were obtained from a cumulative normal function:

$$(2)\quad B_{it} = 1.25 - \int_{-\infty}^{\frac{\text{date}-\mu}{\sigma}} \frac{1}{(\sqrt{2\pi})} \cdot e^{\frac{(\text{date}-\mu)^2}{2\sigma^2}}\, dt \cdot 0.25$$

which was solved using standard normal tables (Snedecor and Cochran 1980). Corrections ranged from 1.25 for censuses conducted prior to any births to 1.00 for censuses conducted after pupping was completed. Except as noted below, censuses were conducted toward the end of the pupping season (8–30 August)

so the correction factors were minor, ranging from 1.000 to 1.062 ($\bar{X} = 1.029$). However, corrections for the 1973 censuses (11–15 June) and the earliest 1988 census (30 May–16 June), which were conducted prior to most births, ranged from 1.249 to 1.250. Corrections for the 1966 censuses of BBAY and FRASERR (27–28 July), which was the middle of the pupping season, ranged from 1.126–1.132.

Since the pupping season is dictated largely by latitude (Bigg 1969b), we assumed that the timing of pupping off the southwest Vancouver Island, Jervis Inlet, and Queen Charlotte Strait was similar to that in the Strait of Georgia, and used equation (2) to adjust counts in these areas to post-pupping levels. Any violation of this assumption would have a minimal effect because the censuses were conducted late (25 August–20 September) in the pupping season such that the corrections were minor, 1.001–1.011.

Quantitative data on the timing of pupping in the Skeena River were not available, but it occurred earlier than in the Strait of Georgia (Bigg 1969b). Fisher (1952) reported that pupping in the Skeena River began in late May, peaked in early June, and was completed by late June. Assuming that the mean pupping date was on day 161 (10 June) and that the duration of the pupping season was equal to that in the Strait of Georgia, we adjusted the Skenna River counts by displacing the Strait of Georgia curve 47 d to the left (Fig. 2). Because the Skeena River censuses were conducted toward the end of the pupping season and on virtually the same date each year, the correction factors were minimal, 1.088–1.106, and had a negligible effect on population trends. The Skeena River pupping curve was also applied to counts for the Queen Charlotte Islands, since the two regions were at a similar latitude. The applicability of the Skeena River curve was substantiated by the recoveries of term-fetuses by seal hunters in the Queen Charlotte Islands (B. and D. McNaughton, General Delivery, Sechelt, British Columbia, V0N 3A0, pers. comm.), which indicated that pupping peaked during 30 May–3 June. Since the Queen Charlotte censuses were conducted after essentially all pups had been born (22–24 July), the correction factor was neglible, 1.001.

The original counts, correction factors, and adjusted counts can be obtained from the senior author.

Trend Analysis

Assuming that the adjusted counts provided an index of absolute seal abundance, we estimated population growth rates for each of the Strait of Georgia subareas, the southwest Vancouver Island and the lower Skeena River from log-linear regressions of the adjusted counts on time. Mean annual finite growth rates, α, were derived from the slopes of the regressions, r, by:

$$(3) \quad \alpha = e^r - 1$$

Adjusted counts for the entire Strait of Georgia in year t, C_{GSt}, were obtained by summing the adjusted counts or estimates of the adjusted counts for the seven subareas:

$$(4) \quad C_{GSt} = \sum_{i=1}^{7} C_{it}$$

Adjusted subarea counts between successive censuses were estimated by interpolating between the preceding and proceeding census counts on a logarithmic scale. Adjusted subarea counts prior to its first census were extrapolated from the earliest count by assuming that the proportion of the population within the subarea had remained constant relative to those subareas that had been surveyed. For example, the NWGULF, which comprised 12.5% of the total Strait of Georgia count when it was first surveyed in 1976, was assumed to have comprised 12.5% of the total count in all years prior to 1976. In effect, equation (4) translated the trends observed in the surveyed subareas to the entire Strait of Georgia.

A population growth rate for the entire Strait of Georgia was estimated from a log-linear regression of $\ln C_{GSt}$ on time. However, in order to minimize the interdependence of the estimated total counts arising from the interpolations in equation (4), each datum was weighted by the proportion of the estimated total count actually counted. Thus, counts that had been purely interpolated had no affect on the regression whereas counts for years in which many subareas had been surveyed exerted the greatest influence.

The shape of the Strait of Georgia population growth curve was evaluated by fitting a stepwise second-order polynomial of $\ln C_{GSt}$ on year, t:

$$(5) \quad \ln C_{GSt} = [a + b \cdot t] + [c \cdot t^2]$$

whereby the first-order term was forced into the regression and the improvement of introducing the second-order term assessed. The procedure was in essence a modification of DeMaster et al's (1982) Dynamic Response Assessment (Boveng 1988). The first term of equation (5) described an exponentially increasing population whereas the second term allowed for depensatory changes in the growth rate with time.

Absolute Abundance

The adjusted counts likely underestimated the actual number of seals present in an area because some animals, particularly those not hauled out, were probably missed during censuses. The actual number of seals in the ith subarea and tth year, N_{it}, was estimated by dividing its corresponding adjusted count, C_{it}, by the estimated proportion of all seals counted during the census, P_{it}:

$$(6) \quad N_{it} = C_{it} \cdot P_{it}^{-1}$$

Independent estimates of the proportion of the actual population counted, P_{it}, were obtained by two methods. The first estimate was made subjectively based on observations of the haulout behaviour of harbour seals in the study area and how this behaviour varied with location (see Results). The second estimate was based on the variability of replicated censuses:

$$(7) \quad P_{it} = \bar{C}_{it} / [C_{\max} + (C_{\max} - C_{\max-1})]$$

where $\bar{C}_{it}$, $C_{\max}$, and $C_{\max-1}$ denote the mean, the highest and the second highest of the replicate counts. Equation (7) embodies as its denominator Robson and Whitlock's (1964) bounded count estimate of the actual population size. The bounded count estimate is based on the premise that each animal in the population has some finite probability of being counted, such that it is theoretically possible, albeit highly unlikely, that all individuals would be counted during a census. Generally, however, the counts represented only a proportion of the actual population, with the proportion depending on tidal conditions, weather, the timing of censuses, the number and competence of observers, etc. If a large proportion of the actual population was being missed during censuses, the inherent variability in the proportion missed would be reflected by large variations in the replicated counts, whereas if only a small proportion was being missed, the replicated counts would be relatively stable.

Population Estimates

The total number of seals on the British Columbia coast was estimated by extrapolating the abundance of seals in areas that had been surveyed to areas that had not been surveyed. Two extrapolations were made. The first was based on the assumption that the density of seals in the surveyed areas was representative of the other areas. Density was expressed in terms of the number of seals per kilometre shoreline. Thus, the total British Columbia population was estimated by extrapolating the density of seals observed in the surveyed areas to the entire coastline of 27 200 km (M. Browning, Canadian Hydrographic Service, Victoria, British Columbia, pers. comm.). Two methods were used to determine relative shoreline lengths: (1) shorelines were traced from 1:525 000 scale nautical charts using a hand-held planimeter; and (2) shorelines were interpolated from 1:1 000 000 digitized maps (World Data Base II). A comparison of the two measures for the 28 coastal Fisheries Statistical Areas indicated good agreement ($r^2 = 0.950$ with slope and intercept not significantly different from 1 and 0, respectively; $P > 0.50$), so the two measures were averaged for subsequent calculations.

The second extrapolation was based on the assumption seals had been hunted for bounty and pelts according to their relative abundance and that recovery rates were uniform throughout the province. The total British Columbia population was estimated by dividing the estimated number of seals off southern Vancouver Island (Strait of Georgia, Jervis Inlet, and the southwest coast of Vancouver Island) by the proportion of the total kill taken from this area. Although we would have preferred to have used the estimated number of seals and relative number of kills in all the areas that had been surveyed, the kill data were not reported in sufficient detail. For example, archival files gave the total number of seals killed in the Queen Charlotte Islands, but there was no way of establishing what proportion of these were taken in the portion of the Queen Charlotte Islands that had been surveyed. Data on the distribution of bounty kills was available for the periods 1927–34 and 1957–60 and of commercial kills for 1963–68 (P. F. Olesiuk and M. A. Bigg, unpubl. data). Several adjustments were made to account for known biases in the kill data. Kills reported for Statistical Area 12 (Johnstone and Queen Charlotte Straits) during 1927–34 were disregarded because large numbers of sea lions had fraudulently been submitted for harbour seal bounties. The proportion of kills in Statistical Area 12 for this period was assumed to have been the same as during 1957–60, 0.081. Bounty kills by two professional seal hunters (B. and D. McNaughton, pers. comm.) during 1957–60 were excluded because they were atypical of other kills, which had been made by many individuals throughout the province on an opportunistic basis. The McNaughtons' kills accounted for 27.5% of all bounty kills made during this period and were concentrated in specific areas. Finally, kills during the last 2 yr of the commercial harvest, 1967–68, were excluded because only small numbers of seals had been harvested and all were taken off southern Vancouver Island.

Results

Trends in Abundance

The similarity of adjusted counts for replicated censuses indicated that they provided a reliable index of abundance. The duplicated counts for BBAY-FRASERR in 1985 and 1986, the SGULF in 1987, and the entire Strait of Georgia in 1988 were within 95.4 to 104.6% of their respective mean counts (mean |deviation| 3.6%). The three duplicated counts of the Skeena River in 1977, 1983, and 1987 were slightly more variable, ranging from 83.2 to 116.8% of their respective mean counts (mean |deviation| 9.6%). The corrections applied to account for unborn pups also appeared to be valid. For example, the adjusted counts in 1973, which were made prior to the pupping season, were similar to the corresponding counts in 1974, which were made toward the end of the pupping season (Fig. 3). In addition, the 1988 adjusted count for the entire Strait of Georgia prior to the pupping season (13 340.4) was similar to the adjusted counts made in the same year toward the end of the pupping season (14 613.7) and during the autumn moult when pupping had been completed (13 662.5).

During the study, seals increased in abundance with time in all areas and subareas (Figs. 3 and 4). Analysis of the Strait of Georgia counts indicated that both the mean number of seals on haulouts and the number of haulouts had increased (Table 1). The correlation coefficients of the log-linear regressions indicated that the increases were statistically significant ($P < 0.05$) in all cases where there were sufficient numbers of counts to make such inferences (Table 2). During 1973–88, the mean annual rates of increase in the seven Strait of Georgia subareas ranged from 8.6 to 24.7% per annum, but the rates within subareas were not always constant. For example, counts for BBAY and FRASERR increased at rates of 12.6 and 12.5% per annum, respectively during 1973–82 ($r^2 = 0.971$ and 0.996 with $n = 4$ and 4 respectively; $p < 0.015$), but subsequently stabilized during 1982–88 ($r^2 = 0.001$ and 0.051 with $n = 8$ and 7, respectively; $p > 0.50$). In contrast, counts for the NWGULF and NEGULF increased steadily at rates of 24.7 and 20.9% ($r^2 = 0.998$ and 0.999, respectively; $P < 0.010$; Table 2), respectively over the course of the study.

Despite the differences among and variations within the subarea rates of increase, the estimated total counts for the Strait of Georgia increased steadily ($r^2 = 0.994$; $t_9 = 38.9$; $P < 0.01$) at a rate of 13.6% during 1973–88. This implied that movements of seals between subareas had occurred. For example, the high rates of increase in the NWGULF and NEGULF, which approached the total birth rate, were likely augmented by the dispersal of seals from subareas such as BBAY and FRASERR, which had stabilized. As a result, the proportion of the total Strait of Georgia population in the NWGULF and NEGULF more than doubled from 16% in 1973 to 28% in 1982 and to 37% in 1988. Shifts in relative distribution of seals were also evident within some subareas. For example, the proportion of seals in GULFISL on sites on the east coast of the Gulf Islands facing the open waters of the Strait of Georgia increased from 39% in 1973 to 43% in 1982 and to 52% in 1988. In general, the apparent changes in distribution within subareas during the study suggested an emigration from the more protected sites and an immigration toward the more exposed sites facing the open waters of the Strait of Georgia.

The actual rate of increase of the population may have been overestimated by the cumulative discovery of haulouts that may have existed but been overlooked in the earlier censuses, but this bias was probably small. For example, the rate of increase in the Strait of Georgia during 1974–88 for only sites that were known to exist by 1974 (1976 for NEGULF) was 10.3%. Thus, even if all of the sites discovered since 1974 had been overlooked in the earlier censuses, the bias accounted for only 3.3% of total rate of increase of 13.6%. In reality, many of the

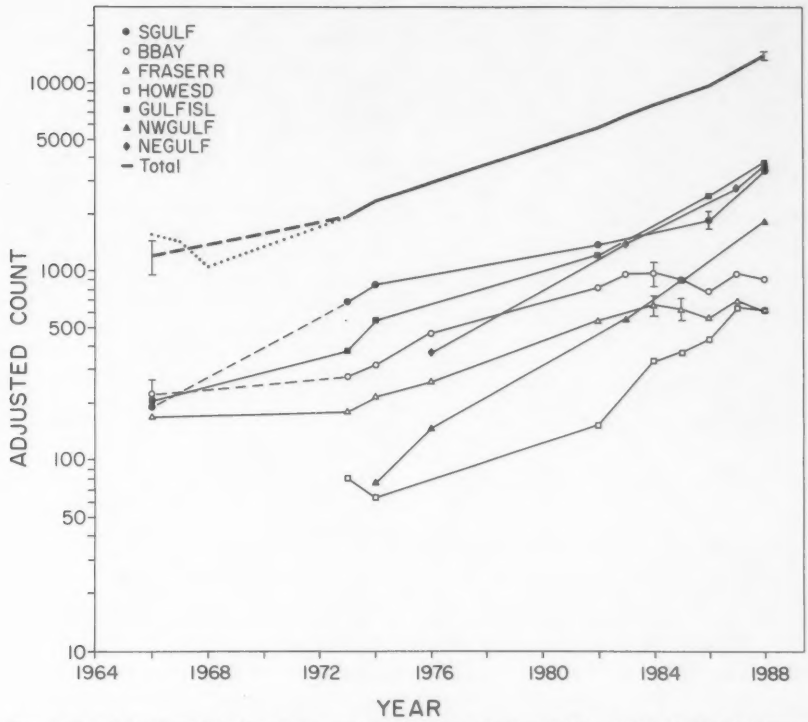

FIG. 3. Trends in adjusted harbour seal counts for the Strait of Georgia during 1966–88. The bold upper line represents the total count for all subareas. The bold dashed segment for 1966–73 is drawn to the midpoint of the potential range of the 1966 estimate (vertical bar) due to the equivocal BBAY and SGULF counts (see text). The bold dotted segment represents the back-calculated size based on kill data. The thin lines represent the trends in each subarea and vertical bars the range of replicated counts. The dashed segment for BBAY during 1966–73 joins the midpoint of the maximum count and the count on the date of the maximum combined BBAY–FRASERR count (see text). The segment for SGULF during 1966–73 is dashed as only a portion of the subarea was censused in 1966.

new sites were in obvious locations and were unlikely to have been missed. Assuming that one-third of the new sites had been missed in the earlier censuses (and two-thirds colonized as a result of the population increase and redistribution), we estimated that the Strait of Georgia population was actually increasing at a rate of 12.5% per annum.

There was no evidence that the rate of increase in the Strait of Georgia declined as the density of seals increased. The trajectory of the increase during 1973–88 conformed closely to a log-linear model ($t_9 = 38.9$; $r^2 = 0.994$; $P < 0.01$) and the fit of the curve was not significantly improved by introducing a second-order term ($t_9 = 0.32$, $P > 0.50$; multiple $r^2 = 0.996$).

Adjusted counts for the Skeena River indicated a mean annual rate of increase of 11.9% during 1977–87 (Fig. 4, Table 2), which was not significantly different from that estimated for the Strait of Georgia ($t_{13} = 0.81$; $P > 0.20$). The increases in the adjusted counts for southwest Vancouver Island between 1976 and 1987 (Fig. 4) represented mean annual rates of increase of 26.5 and 17.2% for BARKLYSD and SWVANISL, respectively, and an overall rate of increase of 20.9%. However, the rates of increase off sothwest Vancouver Island were likely exaggerated by the low counts in 1976, which were made during scattered precipitation and low ceiling. An unsuccessful attempt to census the GULFISL under similar conditions yielded counts that were 66.3% of those made several days later under ideal conditions. Applying the same correction to the 1976 southwest Vancouver Island counts gave rates of increase of 21.9 and 12.9% for BARKLYSD and SWVANISL, respectively, and an overall rate of 16.5%. These data indicated that the rates of increase observed in the Strait of Georgia during 1973–88 were indicative of harbour seal populations in other areas of British Columbia.

The trends in abundance in the Strait of Georgia during 1966–73 were less clear. Few subareas were surveyed in 1966 and the counts were subject to several interpretations. The 1966 censuses of BBAY and FRASERR were each replicated on several days, sometimes during suboptimal haulout conditions. If the maximum counts for each subarea are taken as the most representative, the rate of increase during 1966–73 would have been negligible (Fig. 3). However, the maximum counts for each subarea were obtained on different dates, and recent censuses have indicated that there may be substantial movements between the two subareas over a short time span (P. F. Olesiuk, M. A. Bigg and G. M. Ellis, unpubl. data). If the maximum combined count for both subareas is taken as the most representative, the rate of increase during 1966–73 was 3.6% (Fig. 3). In contrast, the 1966 census of the GULFISL and a partial census of the SGULF (47.5% coverage) indicated

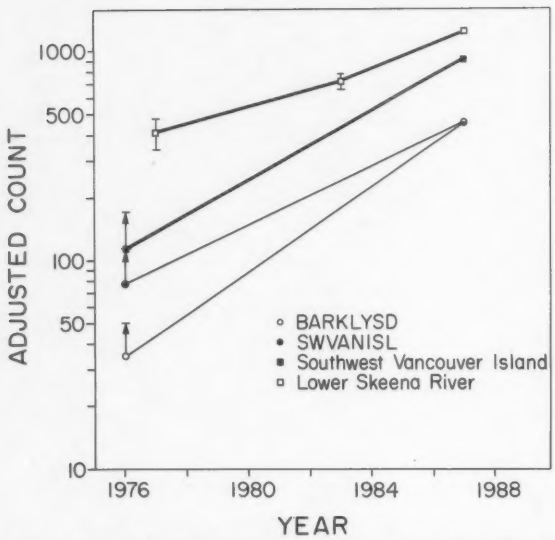

FIG. 4. Trends in adjusted harbour seal counts for the lower Skeena River during 1977–87 and the southwest coast of Vancouver Island during 1976–87. The bold lines represent counts for the total areas and the thin lines counts in each subarea. Vertical bars denote the range of replicated counts. The arrows show the magnitude of corrections to account for the census being conducted during suboptimal conditions (see text).

TABLE 1. Observed changes in number and mean size of known haulouts in the Strait of Georgia between 1973–74 and 1988. Numbers in brackets indicate the number of the known haulout sites occupied during censuses.

	1973–74[a]		1988	
Subarea	Number of haulouts	Mean size	Number of haulouts	Mean size
BBAY	8 (5)	66.3	10 (7)	125.7
FRASERR	9 (7)	44.6	13 (7)	86.3
HOWESD	3 (3)	25.4	12 (8)	76.1
SGULF	34 (28)	28.6	63 (53)	62.3
GULFISL	46 (43)	12.4	91 (78)	46.6
NEGULF	19 (19)	18.6	64 (54)	59.8
NWGULF	8 (8)	9.3	32 (31)	57.1
Total	127 (113)	21.9	285 (238)	59.0

[a]1976 for the NEGULF.

annual rates of increase of 9.4 and 19.8%, respectively during 1966–73 (Fig. 3). Thus, depending on how the data were interpreted, the overall weighted rate of increase during 1966–73 varied from 4.0 (using the maximum individual BBAY and FRASERR counts and excluding the partial census of the SGULF) to 10.6% (using the highest combined BBAY–FRASERR count and incuding the partial census of the SGULF). Furthermore, the 1966 censuses were sometimes conducted during suboptimal haulout conditions and the population was still being hunted. Consequently, a smaller proportion of the population may have been counted and the actual rate of increase exaggerated. Thus, the population growth rate during 1966–73 was likely lower than 1973–88, which was to be expected as some seals were killed for pelts during 1966–68.

Population trends during 1966–73 were also examined by back-calculating the size of the population based on the number of kills during 1966–68. For the back-calculation it was assumed that: (1) maximum productivity of the population was 12.5%; (2) all kills were made just prior to the pupping season; and (3) the number of pelts recovered represented half the number of seals actually killed (Bigg 1969a). The reconstruction predicted an overall rate of increase during 1966–73 of 3.0%, but indicated that the population actually declined during 1966–68 and then increased rapidly during 1969–73 (Fig. 3). In view of the potential biases in the 1966 censuses, we considered that the reconstruction more accurately depicted populations trends in the Strait of Georgia. However, because kills continued in the Strait of Georgia until 1968, and in other areas until only 1966 (P. F. Olesiuk and M. A. Bigg, unpubl. data), this reconstruction was probably not representative of population trends in other areas prior to 1970.

Absolute Abundance

The proportion of the actual number of seals counted in the Strait of Georgia censuses was estimated using two independent approaches. The first estimate, made subjectively in the late 1970s, was predicated on two features of the haulout behaviour of seals in the study area: (1) haulout patterns were highly synchronized among sites during optimal haulout conditions; and (2) the vast majority of seals were hauled out during peak haulout periods in most areas.

Observations during censuses demonstrated the high degree of synchronization in haulout patterns and illustrated the key to obtaining reproducible counts. When censuses were attempted too far in advance of low tide, many seals were observed swimming or milling in the water adjacent to haulouts and the few seals on land were still wet, indicating that they had just recently hauled out. In such cases, we usually landed for 30–45 min before beginning the census, but on one occasion a haulout was continuously circled. Upon our arrival, few seals were hauled out but many were milling nearby. As we circled, the wakes of swimming seals could be seen in the distance as they converged on the haulout. Within about 20 min, seals stopped arriving and most of those that had been milling nearby had hauled out.

As censuses progressed, the pelage of seals dried which made seals more visible from the air. Few animals were observed in the water, except in small estuaries where seals often gathered in groups and rested on the ocean floor and in deep inlets where there were few suitable haulout sites. Toward the end of censuses the pelages of seals became distinctly drier and lighter, which indicated we were approaching the end of the census window. Within an hour or so, seals were easily frightened into the water by the approach of our aircraft or were milling in the water near the haulout when we arrived, perhaps having been frightened before coming within sighting range. At the end of a census, it was not unusual to frighten three to four haulouts in succession whereas seals were rarely frightened earlier in the census.

General observations from boat, land, and aircraft throughout the Strait of Georgia suggested that most seals were hauled out during these peak haulout periods. The proportion of seals on land appeared to vary by area depending on its topography. In areas with numerous tidal islets, reefs, boulders, and sandbars, essentially all seals observed were hauled out. In areas where potential haulout sites were scarce, many swimming seals were observed. Suitable haulout sites were numerous throughut

TABLE 2. Mean annual finite population growth rates calculated from the log-linear regressions fitted to June–August counts for the period 1973–88.

Region/ subarea	Census period	Number of censuses	Correlation Coefficient	Significance level	Rate of increase (%)
		Strait of Georgia			
BBAY	1973–88	12	0.918	<0.001	8.6
FRASERR	1973–88	11	0.947	<0.001	9.4
HOWESD	1973–88	8	0.969	<0.001	16.2
SGULF	1973–88	6	0.954	0.003	9.0
GULFISL	1973–88	6	0.992	<0.001	15.4
NWGULF	1974–88	6	0.998	<0.001	24.7
NEGULF	1976–88	5	0.999	<0.001	20.9
Total[a]	1973–88	11	0.994	<0.001	13.6
		Skeena River			
Total	1973–87	6	0.932	0.002	11.9
		Southwest Vancouver Island			
SWVANISL	1976–87	2	1.000	—	17.2
BARKLYSD	1976–87	2	1.000	—	26.5
Total	1976–87	2	1.000	—	20.9

[a]Based on weighted regression (see text).

most of the Strait of Georgia. Weighing the availability of suitable haulout sites by the density of seals in representative areas, we subjectively estimated that an overall 85–90% of seals in the Strait of Georgia were hauled out during peak haulout periods.

Observations made during censuses also indicated that the vast majority of seals were on land during the peak haulout periods. Had appreciable numbers of seals remained in the water, we would have expected to see more swimming animals. For example, on one occasion when we unsuccessfully attempted to count seals during a light rain, there were noticeably many more than the usual number of seals in the water. However, the number observed on land was only 34% less than that seen several days later when the census was repeated under ideal conditions. The fact that a 34% decline in the number hauled out resulted in a pronounced increase in the number of swimming seals implied that much less than one-third of the population was in the water during normal census conditions.

A second estimate of the proportion of the actual population counted was made more recently from replicated censuses using the bounded count method (Robson and Whitlock 1964). Duplicated censuses of BBAY–FRASERR in 1984 and of the SGULF in 1986 were considered true replicates as they had been conducted within 4–15 d of each other. The estimated mean proportion of the population counted in these censuses ranged from 0.893 to 0.905 (Table 3). The bounded count method was also applied to the censuses in the Strait of Georgia made prior to and toward the end of the pupping season in 1988, as well as to the portion surveyed a third time during the autumn moult. Although some of the variability in these counts may have been due to inaccuracies in the corrections made for unborn pups or movements of seals during the 4 mo between censuses, these counts indicated that the mean counts represented 0.848 to 0.880 of the actual population (Table 3). Because the above estimates were based on only two to three replicates, each was subject to considerable bias, the potential of which increases as a function of n^{-2} (Robson and Whitlock 1964). In an attempt to improve sample sizes, the bounded count method was applied to the eight censuses of BBAY–FRASERR in August during 1982–88, over which period the population had been stable ($r^2 = 0.024$; $P > 0.50$). These counts indicated that the mean count represented 0.878 of the actual population (Table 3). Averaging the bounded count estimates, we estimated that the Strait of Georgia counts typically represented 0.884 of the actual population.

We suspected that a larger proportion of seals was missed in the censuses outside the Strait of Georgia. With the exception of Jervis Inlet, which accounted for only a small portion of the total area surveyed, the surveyed areas offered numerous potential haulout sites. Observations during censuses indicated that haulout patterns were basically the same as those in the Strait of Georgia. However, since these areas had not been studied as intensively it was possible that some haulout sites were missed or that there were subtle differences in haulout patterns in these areas. The bounded count estimates for the three duplicated censuses of the Skeena River in 1977, 1983, and 1987 indicated that the mean counts represented 0.665–0.916 ($\bar{X} = 0.790$) of the actual population (Table 3). We assumed that the counts for the remaining areas outside the Strait of Georgia represented 0.80 of the actual population.

Population Estimates

Synthesizing the results in the two preceding sections, we estimated that actual post-pupping population in the Strait of Georgia increased from 2170 in 1973 to 15 810 in 1988. At its lowest point in the late 1960s, the Strait of Georgia population was probably on the order of 1200. Similarly, the post-pupping population in the lower Skeena River was estimated to have increased from 520 in 1977 to 1590 in 1987 and the population off southwestern Vancouver Island from 210 in 1976 to 1130 in 1987. The post-pupping population on the north and east coast of the Queen Charlotte Islands was estimated to have been 2450 in 1986, the Jervis Inlet population 290 in 1987, and the entrance to the Queen Charlotte Strait population 580 in 1988.

The total number of seals on the British Columbia coast was estimated based on the density of seals in the surveyed areas. The six surveyed areas comprised 6518 km, or 24.0% of the total British Columbia coastline (Table 4). Their total combined population, adjusted to 1988 levels (1987 estimates were

TABLE 3. Estimates of the actual population of seals (N) and the proportion of seals counted during censuses (P) using the bounded count method for replicated censuses.

Subarea(s) censused	Census period	Number counts	Adjusted counts max	Adjusted counts max − 1	Mean count	N	P
			Strait of Georgia				
BBAY–FRASERR	Aug. 84	2	1688.7	1567.0	1627.9	1810.4	0.899
BBAR–FRASERR	Aug. 85	2	1591.9	1484.3	1538.1	1699.5	0.905
SGULF	Aug. 86	2	1943.7	1794.1	1868.9	2093.3	0.893
Complete	May–Aug. 88	2	14613.7	13340.4	13977.1	15887.0	0.880
Partial[a]	May–Sept. 88	3	6495.8	6073.0	6284.4	6918.6	0.848
BBAY–FRASERR	Aug. 1982–88	8	1688.7	1646.4	1519.8	1731.0	0.878
Mean							0.884
			Skeena River				
Complete	Jun. 77	2	475.9	339.0	407.5	612.8	0.665
Complete	Jun. 83	2	775.5	648.4	712.0	902.6	0.789
Complete	Jun. 87	2	1294.2	1217.2	1255.7	1371.2	0.916
Mean							0.790

[a]Based on portion of region surveyed in September: BBAY, FRASERR, HOWESD, 46.3% of SGULF, 22.6% of GULFISL, and 47.6% of NEGULF.

TABLE 4. Density of seals (seals · km^{-1}) in surveyed areas of British Columbia adjusted to 1988 levels.

Area	Population	Shoreline	Density
Strait of Georgia[a]	16 134.6	3221.1	5.01
Lower Skeena River	1 788.2	345.9	5.17
SW Vancouver Island	1 276.2	556.8	2.29
NE Queen Charlotte Islands	3 105.5	2093.6	1.48
Queen Charlotte Strait	577.3	300.2	1.92
Overall	22 881.8	6517.6	3.51

[a]Includes Jervis Inlet.

multiplied by 1.125 and 1986 estimates by 1.125^2) was estimated at 22 882 seals, which represented a mean weighted density of 3.51 seals · km^{-1}. Applied to the entire British Columbia coast of 27 200 km, the total post-pupping population was estimated to have been on the order of 95 500. However, this was probably an overestimate because the density of seals in the Strait of Georgia and Skeena River, which represented a large proportion of the total area surveyed, appeared to be higher than in other areas of the province (Table 4).

A second estimate of the total number of seals in British Columbia was derived from the relative distribution of kills. During 1927–34, 1957–60, and 1963–66, the waters off southern Vancouver Island accounted for 18.3, 21.4, and 21.6% of the total seal kill in British Columbia (P. F. Olesiuk and M. A. Bigg, unpubl. data). The proportion of the annual kill taken from this area was remarkably constant, ranging from 15.2 to 26.1%, except for 1928–29 when this area accounted for only 7.6% of the total kill. The total post-pupping population off southern Vancouver Island in 1988 was estimated to have been 17 410. Assuming that seals were hunted according to their relative abundance and that recovery rates were uniform, we estimated that the total British Columbia poulation in 1988 was on the order of 80 600–95 140 ($\bar{X}$ = 85 700).

To gain further insight into potential biases in the density and kill data, we examined the relative distribution of kills in broad regions of British Columbia (e.g. the west coast Vancouver Island, the central mainland coast). The density of kills per kilometre of shoreline in each region, which presumably provided an index of the density of seals, was compared with the density of kills in the Strait of Georgia, where the density of seals was known. Densities of kills on the west coast of Vancouver Island, in Johnstone and Queen Charlotte Straits, and on the central mainland coast were about 30–40% of the density of kills in the Strait of Georgia. This suggested that the densities of seals observed in the censuses off southwestern Vancouver Island and the entrance to Queen Charlotte Strait (Table 4) were probably representative of these broader regions. Similarly, the density of kills for the Skeena and Nass Rivers and surrounding areas (Department of Fisheries and Oceans, Statistical Areas 3 and 4) were of the same magnitude as in the Strait of Georgia, which suggested that the density of seals observed in the lower Skeena River (Table 4) was representative of the surrounding area. In contrast, the density of kills in the Queen Charlotte Islands was almost equal to that in the Strait of Georgia, whereas the density observed on the south and east coasts of the Queen Charlottes (Table 4) was only about 30% of the density observed in the Strait of Georgia. This implied that the density of seals in the unsurveyed portions of the Queen Charlotte Islands was much greater than in the surveyed portions or that many seals had been missed in the Queen Charlotte Island censuses. Alternatively, the density of seals in the Queen Charlotte Islands, relative to other regions, may have declined since the bounty and commercial kills were taken. In support of the latter, the Queen Charlotte Islands were hunted intensively by B. and D. McNaughton (pers. comm.) during the late 1950s and early 60s. Their logbooks for 1955–60 indicated that 2074 seals had been taken in this area, which may have depleted the Queen Charlotte Island population to a greater degree than other regions of British Columbia.

Based on the foregoing assessment, we derived a stratified estimate of the total British Columbia population. The density of seals observed in the Strait of Georgia was applied to the shoreline in this area, the density of seals observed off southwestern Vancouver Island and in the entrance to Queen Charlotte Strait was applied to the west coast of Vancouver Island, Johnstone and Queen Charlotte Straits, and the central mainland coast shorelines, and the density observed in the lower

Skeena River was applied to the Skeena and Nass Rivers and adjacent areas. This gave a total post-pupping population estimate of 75 000–88 000, depending on what density was applied to the Queen Charlotte Islands. We considered this the best estimate given all the available data.

Assuming that the population trends in the Strait of Georgia were representative of populations throughout British Columbia, the total post-pupping population was estimated to have been on the order of 9000–10 500 when the species was protected in 1970.

Discussion

Aerial censuses have been widely used to assess harbour seal abundance and population trends (Johnson and Jefferies 1977; Mate 1977; Calambokidis et al. 1979; Everitt and Braham 1980; Stewart 1982; Jefferies 1986; Harvey 1987). Owing to the difficulty in counting seals in the water, censuses have usually been timed to coincide with peak haulout periods. However, there has been no consensus as to when peak haulout periods occurred or the proportion of seals on land during these peaks.

The haulout patterns of harbour seals have been shown to be influenced by many factors including tidal cycles, time of day, season, and weather conditions (Venables and Venables 1955; Rosenthal 1968; Schneider and Payne 1983; Stewart 1984; Yochem et al. 1987; Harvey 1987). Haulout patterns within our study area were dictated predominantly by tidal levels. Many of the haulout sites utilized were exposed only at low tides and in some areas, such as BBAY, no suitable haulout substrates were available at high or even moderate tides. Consequently, the haulout patterns of seals were synchronized to coincide with the low tide, as has been observed elsewhere (Schneider and Payne 1983). In contrast, haulout patterns in some other regions appeared to be dictated mainly by time of day (Stewart 1984; Godsell 1988). For example, in California where tidal fluctuations are much less pronounced than in British Columbia, the number of seals on land peaked during early afternoon irrespective of tide levels (Stewart 1984), but haulout patterns were not nearly as synchronized (Yochem et al. 1987).

In our study area, greater numbers of seals generally hauled out on morning low tides than on afternoon low tides. We suspected that fewer seals came ashore or that haulout bouts were shorter when it was hot. Harvey (1987) noted that the maximum number of seals hauled out on morning low tides in protected areas and on afternoon low tides in exposed areas, and suggested the differences were due to the frequency of disturbances. In most studies, the largest number of seals on land was reported during either the pupping season (Rosenthal 1968), the annual moult (Stewart and Yochem 1984), or both (Jefferies 1986). We found that counts made just prior to the pupping season (adjusted to post-pupping levels), toward the end of the pupping season, and during the autumn moult were consistent, provided that a large enough area was surveyed. However, seasonal movements were evident between these censuses. Localized censuses could therefore give the false impression that numbers on land had changed. Thus, the timing of haulout peaks and the proportion of seals hauled out during these peaks varies depending on local conditions.

Several studies have used telemetry to estimate the proportion of seals hauled out at a given time (Pitcher and McAllister 1981; Stewart and Yochem 1983; Allen et al. 1987; Yochem et al. 1987; Harvey 1987). In most studies, the capture sites were monitored for radio-tagged seals at daily peak haulout times or continuously. In some cases, the proportion of seals on land was estimated for a subsample of seals considered to be resident to the capture site or adjacent areas were also periodically monitored to account for the dispersal of tagged seals from the capture site. Boveng (1988) reviewed the telemetry studies and concluded that 0.5–0.7 seals were typically on land during peak haulout times. This was substantially less than our estimate of 0.790–0.884. The discrepancy may partly be due to regional variations in haulout behaviour. Perhaps more importantly, because the telemetry studies involved small sample sizes, data were pooled over time, usually by month. Consequently, these estimates represented the proportion of seals on land during typical daily haulout peaks. In contrast, our censuses were conducted during optimal haulout conditions, which occurred on only a few days each month.

The annual rate of increase of 12.5% estimated for the British Columbia population was similar to rates reported in other areas of the northeast Pacific. Non-pups and pups were estimated to have increased at rates of 10.7 and 19.1%, respectively in the Columbia River and adjacent waters during 1976–82 (Jefferies 1986); populations at a rate of 6–9% on the Oregon coast during 1975–83 (Brown 1986; Harvey 1987); 14.7% on mainland California during 1965–86 and 9.1% on the Channel Islands during 1975–86 (Boveng 1988). In other regions, annual rates of increase of 11.9% were reported for a haulout at Stage Point, Massachusetts, during 1972–83 (Payne and Schneider 1984), and 13% in the Kattegat-Skagerrak during 1979–86 (Heide-Jorgensen and Harkonen 1988).

The recent increases in British Columbia and in some other regions follow periods of control or commercial kills, and thus probably represent the recovery of populations. Reconstructions of historic trends in British Columbia based on the number of kills (P. F. Olesiuk and M. A. Bigg, unpubl. data) indicated that the population was reduced in size by large bounty kills during the 1950s and early 1960s. The depleted population could not sustain the large commercial harvests during 1963–69 and declined precipitously. However, the population reconstruction indicated that the British Columbia population has attained, and perhaps exceeded, historic levels in the last several years.

The population growth rate of 12.5% was probably close to the maximum intrinsic rate for this species. Given that the total birth rate was 25% (Bigg 1969a), the 12.5% rate of increase implied that the mean annual mortality rate for all age-classes was 10.0% (i.e. $1.25 \cdot [1–0.10] = 1.125$). Estimates of adult mortality rates for harbour seals from age-composition data range from 12–20% (Bigg 1969a; Boulva and McLaren 1979; Pitcher and Calkins 1979), but these probably tended to be overestimates because the populations sampled had been exploited or were increasing. Natural mortality rates on the order of 10% have been reported for other adult phocids (e.g. Lett et al. 1981), and it was generally considered that juveniles exhibited higher mortality.

Although populations in British Columbia were estimated to have increased almost 10-fold during 1970–88 and may have been approaching historic levels, there was no evidence that the population growth rate declined in recent years. The reduced rates of increase in areas that had apparently realized their carrying capacity, such as Boundary Bay and the Fraser River, appear to have been compensated by dispersal to other areas, such as the northeastern and northwestern Strait of Georgia. The increases reported in some other regions, such as Massachusetts and California, may also have been due to the dispersal

of seals from neighboring areas (Payne and Schneider 1984; Boveng 1988). However, the overall growth of the British Columbia population cannot be attributed to immigration, as populations were increasing in both northern and southern British Columbia and in Washington State to the south (Calambokidis et al. 1985).

Obviously, the population growth cannot continue indefinitely. Fowler (1981a,b) argued that for large mammals, density dependence was expressed at population levels close to carrying capacity, such that inflections in their population growth curves occurred shortly before populations attained their carrying capacity. Moreover, the population growth curves would be further skewed by any time lags in depensatory mechanisms, such that it is possible that populations may temporarily exceed their carrying capacity. We anticipate that continued monitoring of harbour seal trends in British Columbia will provide insight into the mechanisms of population regulation.

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Sampling Littoral Fish with a Seine: Corrections for Variable Capture Efficiency[1]

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Pierce, C. L., J. B. Rasmussen, and W. C. Leggett. 1990. Sampling littoral fish with a seine: corrections for variable capture efficiency. Can. J. Fish. Aquat. Sci. 47: 1004–1010.

Capture efficiency of a beach seine varies greatly depending on aspects of the littoral zone habitat and fish community. To address this sampling bias, we quantified seine efficiency and several habitat and fish community variables at 26 littoral stations in 10 southern Quebec lakes. We then generated regression models predicting capture efficiencies for total, midwater, and benthic fish. Predictions from these models yield "sliding" correction factors for seine catches. Bottom snags and seine rolling generally reduced capture efficiencies, and higher proportions of benthic fish were associated with reduced capture efficiencies for total fish. Higher macrophyte biomass was associated with increased capture efficiencies. Fish size was a significant predictor of capture efficiency only for benthic fish; smaller fish escaped the seine more readily. Regression models explained 26–73% of the observed variation in capture efficiency. Use of our models will improve the accuracy of abundance estimates from littoral seining with little additional effort.

Le rendement de capture d'une seine de rivage varie énormément selon les caractéristiques de l'habitat riverain et de l'ichtyofaune. Pour corriger ce biais dans l'échantillonnage, nous avons quantifié le rendement de capture d'une seine et plusieurs variables relatives à l'habitat et à l'ichtyofaune à 26 stations riveraines dans 10 lacs du sud du Québec. Nous avons ensuite produit des modèles de régression permettant de prévoir les rendements de capture pour l'ensemble des poissons, pour les poissons nageant entre deux eaux et pour les poissons benthiques. Les prévisions obtenues à l'aide de ces modèles ont donné des facteurs de correction pour les captures variant selon les conditions. Les obstacles rencontrés au fond de l'eau et le fait que la seine roule diminuent généralement les rendements de capture. Quand les rendements de capture pour l'ensemble des poissons étaient bas, la proportion de poissons benthiques était élevée. Par ailleurs, quand les rendements de capture étaient élevés, la biomasse de macrophytes l'était également. La taille des poissons était un bon facteur de prévision du rendement de capture seulement pour les poissons benthiques; en effet, les poissons plus petits peuvent s'échapper du filet plus facilement. Les modèles de régression nous ont permis de rendre compte de 26–73 % de la variation du rendement de capture observée. Nos modèles permettront d'améliorer la précision des estimations d'abondance effectuées au moyen d'un échantillonnage à la seine près de la rive moyennant peu de travail supplémentaire.

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Beach seining is a common method for assessing abundance and species composition of littoral zone fish communities and has been used widely in freshwater, marine, and estuarine studies (Nielson and Johnson 1983). Seining combines several advantages over other assessment techniques: (1) no poisons or explosives are used, (2) the gear is simple and easy to deploy, (3) sampling is rapid, (4) a large area can be sampled, (5) the limits of the sampling area are precisely defined, and thus habitat attributes can be accurately quantified, (6) sampling is active and, in principle, should capture all species equally well, (7) fish are obtained live, with minimal trauma, and are collected soon after capture, enabling accurate assessment of gut contents, temporal distribution patterns, and providing for live release if necessary.

Despite these advantages, a major source of bias presently limits the effectiveness of seining for quantitative sampling. Physical obstructions such as rocks, macrophytes, logs, tree branches, and moorings interfere with the seine and prevent it from passing through the entire water column. The process of snagging and unsnagging from obstructions can provide an escape route for enclosed fish, and dense macrophyte growth can cause the seine to roll up from the bottom into a tight coil. In addition, benthic fish may be more likely to escape than fish higher up in the water column. These problems have been recognized in previous studies (Richkus 1980; Frankiewicz et al. 1986; Lyons 1986; Parsley et al. 1989) and fixed, species-specific correction factors have been proposed. However, variability of habitat and fish community factors results in widely varying degrees of sampling bias and existing corrections are not sensitive to this variation.

The purpose of this study was to quantify relationships of habitat and fish community characteristics with capture efficiency of a beach seine for sampling littoral fish, and to generate predictive models of seine efficiency. Predictions from these models yield correction factors for abundance estimates obtained from seine catches, taking into account the variation in efficiency due to differences in littoral habitats and fish communities.

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TABLE 1. Habitat classifications (M = midwater, B = benthic) and relative abundances of fish species captured in this study (all stations combined).

Species	Habitat	Percent of total catch		Length range (mm)
		Number	Mass	
Perca flavescens	M	27	37	48–269
Notemigonus crysoleucas	M	26	9	51–199
Lepomis gibbosus	M	19	18	32–190
Fundulus diaphanus	B	7	2	49–83
Pimephales notatus	M	4	<1	50–81
Etheostoma olmstedi	B	4	<1	48–82
Catostomus commersoni	B	4	12	50–435
Micropterus dolomieui	M	2	<1	39–199
Semotilus corporalis	M	2	<1	56–178
Ictalurus nebulosus	B	1	2	42–267
Notropis cornutus	M	1	<1	50–126
Ambloplites rupestris	M	1	2	35–224
Esox niger	M	<1	6	88–498
Semotilus atromaculatus	M	<1	<1	50–95
Percina caprodes	B	<1	<1	50–66
Micropterus salmoides	M	<1	<1	47–107
Esox lucius	M	<1	7	106–670
Notropis hudsonius	M	<1	<1	65–83
Osmerus mordax	M	<1	<1	124–142
Moxostoma anisurum	B	<1	1	433 (1)

TABLE 2. Habitat and habitat-related variables at 26 sampling stations included in this study.

Lake	Station	Depth (m)	Mean macrophyte biomass ($g \cdot m^{-2}$)	Bottom	Snags	Seine rolling
Brome	1	0.6	442	Smooth	0	None
	2	0.6	889	Smooth	0	Slight
Bromont	3	0.7	0	Smooth	0	None
	4	1.2	3945	Medium	0	Slight
Brompton						
North end	5	0.9	1379	Medium	0	None
	6	0.8	1083	Smooth	0	None
Boat landing	7	2.5	1437	Medium	3	None
	8	2.0	744	Medium	0	None
Pointe Rocheuse	9	2.2	62	Rough	2	None
	10	2.1	0	Rough	8	None
d'Argent	11	0.7	412	Smooth	0	Slight
	12	0.7	0	Smooth	0	None
Hertel	13	1.4	357	Rough	4	None
	14	1.8	223	Rough	3	None
Magog	15	1.0	2395	Smooth	0	None
	16	1.0	2359	Smooth	0	None
Massawippi	17	1.0	1227	Smooth	0	Slight
	18	1.5	819	Smooth	0	None
Memphremagog						
Macpherson Bay	19	1.7	576	Rough	5	None
	20	1.4	2649	Smooth	1	None
Newport Bay	21	1.0	3026	Smooth	0	Moderate
	22	1.0	4341	Smooth	0	Severe
Roxton Pond	23	0.7	1416	Smooth	0	None
	24	0.9	1331	Smooth	0	None
Waterloo	25	1.2	1705	Smooth	0	Slight
	26	1.0	0	Smooth	0	None

Materials and Methods

Study Sites and Littoral Fish Communities

Our study was conducted in 10 lakes located in the Eastern Townships region of southern Quebec, Canada (45°N, 72°W). Limnological attributes of these lakes are described by Rasmussen (1988) and littoral fish communities are described by Boisclair and Leggett (1989). Yellow perch (*Perca flavescens*), golden shiner (*Notemigonus crysoleucas*), and

TABLE 3. Fish community variables at 26 sampling stations included in this study. — indicates no benthic fish captured.

Station	Mean fish mass (g wet)			Benthic proportion[a]	
	Total	Midwater	Benthic	Density	Biomass
1	5.00	7.45	2.13	0.41	0.18
2	2.44	3.00	2.18	0.65	0.60
3	3.40	4.23	2.63	0.52	0.41
4	46.03	33.87	68.64	0.35	0.52
5	6.56	6.57	3.64	0.003	0.001
6	11.08	11.08	—	0	0
7	10.07	9.97	29.10	0.005	0.01
8	7.29	7.29	—	0	0
9	3.26	3.26	—	0	0
10	2.66	2.66	—	0	0
11	3.87	3.53	15.93	0.03	0.11
12	5.02	5.02	—	0	0
13	10.69	10.69	10.66	0.03	0.03
14	6.61	6.61	—	0	0
15	21.74	14.61	75.01	0.12	0.41
16	31.04	32.98	8.72	0.08	0.02
17	3.13	3.19	3.05	0.46	0.45
18	33.70	42.28	25.58	0.50	0.04
19	1.86	1.86	1.89	0.06	0.05
20	10.51	6.44	16.32	0.41	0.64
21	2.24	2.03	3.54	0.14	0.22
22	3.37	3.36	3.39	0.20	0.20
23	13.75	13.92	8.86	0.03	0.02
24	18.93	15.06	65.44	0.08	0.27
25	7.57	7.11	29.46	0.02	0.08
26	17.74	13.48	113.27	0.04	0.27

[a]Proportion of benthic fish to total fish captured in all seine hauls.

pumpkinseed (*Lepomis gibbosus*) are the dominant littoral fish species in these lakes (Table 1). We sampled at 26 different littoral zone stations varying widely in several habitat and habitat-related variables (Table 2). Substrate conditions at these stations ranged from smooth sand to large rocks, and macrophyte biomass ranged from none to extremely dense. The dominant macrophyte species at most sites were *Vallisneria americana*, *Myriophyllum spicatum*, *Elodea canadensis*, *Potamogeton robinsii*, and *P. crispus*, with several other species occurring less frequently. Maximum sampling depths ranged from 0.6 to 2.5 m. Mean fish size ranged several-fold, and the proportion of benthic fish in communities varied from 0 to over 50% (Table 3). The range of habitats and fish communities included in our study represent most of the conditions encountered in northern temperate zone lakes.

Capture Efficiency Estimates

We estimated capture efficiency of a beach seine at each station in late summer 1987 by repeated removal sampling from an area completely enclosed by a block net. We used a 52×2.6 m knotless nylon (6-mm mesh) beach seine, with a continuous lead-core bottom line, plastic floats along the top line, and a 2.6 m^3 bag in the center. The seine was deployed from a small boat in a semicircle extending out from the shoreline, enclosing an area of 430 m^2. Fish generally seemed to ignore the boat until it came to within 2 or 3 m, so we assumed that evasion or other movements into or out of the enclosed area during seine deployment were negligible. We then placed a block net of similar material and construction (but of slightly larger dimensions and without a bag) just outside of and as close to the beach seine as possible. The seine was pulled to shore, capturing a percentage of the enclosed fish, while the block net remained in place and prevented movement of fish between the enclosed and adjacent unenclosed areas. We then replaced the seine just inside the block net and pulled it to shore, repeating this process two or three times depending on how rapidly catches declined with each subsequent haul. Finally, the block net was pulled to shore, constituting the final (fourth or fifth) haul. All fish were put on ice immediately and frozen within a few hours. In the laboratory, fish were identified, measured (TL) to the nearest millimetre, and weighed (wet) to the nearest 0.01 g. We used the Zippin (1958) method to estimate absolute abundance (calculated as both density and biomass) for all fish together (total fish), midwater fish, and benthic fish. (Fish habitat classifications were based on descriptions by Scott and Crossman (1973) and personal observations.) Abundance estimates were not calculated in cases where catch in the first haul was less than 10 individuals. Capture efficiency was then calculated as

$$(1) \quad E_D \text{ or } _B = C_1 \cdot A^{-1},$$

where E is the estimated efficiency (for sampling density or biomass), C_1 is the catch in the first haul, and A is the estimated absolute abundance.

For comparison with these removal estimates, we made simultaneous efficiency estimates based on the percentage of recaptures of marked fish introduced to the enclosed area. Yellow perch, pumpkinseed, and golden shiner were obtained from nearby areas, marked with small fin clips, and 10–20 of each were released inside the seine before the first haul. Capture efficiency for the combined group of marked fish was then calculated as

$$(2) \quad E_R = R_1 \cdot M^{-1},$$

where E_R is the estimated efficiency, R_1 is the number of marked fish recaptured in the first haul, and M is the number of marked fish released inside the seine.

Habitat and Habitat-Related Variables

We quantified littoral habitat and habitat-related variables before and during the seining at each station. Maximum depth was recorded inside the enclosed area. Prior to pulling the seine, a diver sampled submerged macrophytes by taking four 740-cm^2 quadrats at each of three (deep, middle, and nearshore) locations within the enclosed area. Macrophytes were spindried in the laboratory, weighed, and the mean biomass at each station was calculated. While sampling macrophytes, the diver subjectively categorized the bottom as smooth (sand or muck, little or no organic debris), medium (sand or muck mixed with gravel and pebbles, a few cobles and/or tree branches), or rough (numerous cobbles and boulders, tree branches, logs).

We also quantified two habitat-related variables pertaining to the mechanical performance of the seine. As the seine was pulled to shore on the first haul, we recorded the number of times the bottom line became snagged on obstructions and had to be freed by pulling upward on the seine from the boat. Pulling the seine through dense macrophyte stands frequently caused it to roll up from the bottom. We categorized the degree of seine rolling as none, slight (1–25% of the height of the seine), moderate (25–50%), or severe (>50%).

Statistical Analyses

We used linear regression to analyze relationships between capture efficiency and the habitat and fish community variables

TABLE 4. Capture efficiency estimates for total, midwater, benthic, and marked fish. E_D (density) and E_B (biomass) are efficiency estimates derived from removal sampling, and E_R are efficiency estimates derived from recaptures of marked fish. — indicates insufficient data to estimate efficiency.

	Total fish		Midwater fish		Benthic fish		Marked fish[a]
Station	E_D	E_B	E_D	E_B	E_D	E_B	E_R
1	0.86	0.95	0.90	0.98	0.81	0.82	1.0
2	0.42	0.52	0.34	0.57	0.45	0.49	0.90
3	0.53	0.77	0.77	0.90	0.31	0.56	1.0
4	0.59	0.96	0.72	0.95	0.33	0.98	0.89
5	0.91	0.97	0.91	0.97	—	—	1.0
6	0.96	0.99	0.96	0.99	—	—	0.97
7	0.92	0.97	0.93	0.98	—	—	0.64
8	—	—	—	—	—	—	0.88
9	—	—	—	—	—	—	0.77
10	0.10	0.07	0.13	0.08	—	—	0.39
11	0.65	0.68	0.65	0.72	0.91	0.35	0.98
12	0.80	0.70	0.78	0.58	—	—	1.0
13	0.60	0.81	0.62	0.82	—	—	0.65
14	0.92	0.77	0.92	0.77	—	—	0.81
15	0.65	0.97	0.69	0.96	—	—	0.69
16	0.84	0.96	0.90	0.98	—	—	0.86
17	0.75	0.83	0.82	0.88	0.66	0.78	0.82
18	0.81	0.99	0.94	0.99	0.68	0.98	0.88
19	0.56	0.52	0.58	0.55	—	—	0.40
20	0.62	0.89	0.61	0.87	0.64	0.91	0.93
21	0.79	0.78	0.87	0.88	0.17	0.34	0.44
22	0.55	0.58	0.61	0.64	0.17	0.33	0.35
23	0.87	0.96	0.89	0.98	—	—	0.93
24	0.81	0.96	0.80	0.96	—	—	0.93
25	0.91	0.96	0.92	0.97	0.33	0.89	0.85
26	0.83	0.94	0.84	0.97	—	—	0.90

[a]Efficiencies calculated from combined recaptures of yellow perch, pumpkinseed, and golden shiner.

TABLE 5. Predictive models for capture efficiency of a beach seine. E_P = predicted efficiency, R^2 = proportion of variance explained by the model, RMS = residual mean square error.

		Significance (P)				
Group	Model	Coefficient	Model	R^2	RMS	n
	Density					
Total fish	(1) arcsine $E_P = +1.129$	<0.001	<0.001	0.52	0.203	24
	-0.092 Snags	<0.001				
	-0.567 arcsine (Benthic proportion)$^{0.5}$	0.001				
Midwater fish	(2) arcsine $E_P = +0.972$	<0.001	0.011	0.26	0.255	24
	-0.071 Snags	0.011				
	Biomass					
Total fish	(3) arcsine $E_P = +1.348$	<0.001	<0.001	0.73	0.196	24
	-0.107 Snags	<0.001				
	-0.265 Seine rolling	<0.001				
	$+0.075$ $\log_{10}$ Macrophyte biomass	0.003				
Midwater fish	(4) arcsine $E_P = +1.372$	<0.001	<0.001	0.68	0.214	24
	-0.112 Snags	<0.001				
	-0.240 Seine rolling	0.001				
	$+0.069$ $\log_{10}$ Macrophyte biomass	0.011				
Benthic fish	(5) arcsine $E_P = +1.051$	<0.001	0.007	0.71	0.243	11
	$+0.012$ Mean benthic fish mass	0.013				
	-0.223 Seine rolling	0.026				

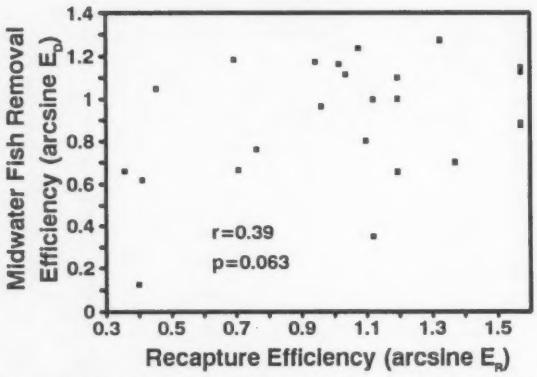

FIG. 1. Relationship of capture efficiency estimates for density of midwater fish with simultaneous estimates from combined recaptures of marked yellow perch, pumpkinseed, and golden shiner.

presented in Tables 2 and 3. Variables were transformed as necessary to conform with assumptions and conventions of linear regression, and only independent variables with significant regression coefficients (H_0: $b=0$) were retained in the final models. We used the simple arcsine transformation for efficiencies rather than the customary angular transformation (arcsine ($p^{0.5}$)) due to the skewed distribution of the raw efficiency estimates. Seine rolling categories were coded as follows: none = 0, slight = 1, moderate = 2, severe = 3. Correlation coefficients and condition indices (Belsley et al. 1980) were examined to insure that our models contained no serious multicollinearities. All analyses were performed using the REG and CORR procedures of SAS (Luginbuhl et al. 1987).

Results and Discussion

Littoral Habitat and Seine Operation

Habitat conditions varied widely across the 26 stations we sampled (Table 2), resulting in variable mechanical performance of the seine. Snagging was much more frequent at stations with rough bottoms than smoother ones, averaging 4.4 snags per haul compared with 0.6 and 0.06 snags per haul at medium and smooth stations, respectively. Seine rolling was positively related to macrophyte biomass, although single observations in the severe and moderate rolling categories limit this interpretation. Macrophyte biomass averaged 909 and 1635 $g \cdot m^{-2}$ at stations where rolling was scored as none and slight, and was 3026 and 4341 $g \cdot m^{-2}$ at the stations where rolling was moderate and severe, respectively.

Direct observations by a diver during seining confirmed these relationships between the habitat and seine performance. Snagging of the bottom line on rocks, branches, and logs was commonly observed, and it was necessary in a few instances for the diver to assist in unsnagging the seine. Seine rolling occurred when dense, firmly rooted macrophytes bent over in front of the advancing bottom line, forming a thick mat over the bottom. Both snagging and rolling caused the bottom of the seine to lift off the substrate, providing a potential escape route for the entrapped fish. Seine rolling can also pull the top line under the surface, allowing additional opportunity for escape. Extra care is necessary to quantify these factors when working in difficult conditions.

Capture Efficiency Estimates

Catches generally declined rapidly with repeated seining and usually approached zero on the last haul. A previous study in which ponds were repeatedly seined followed by draining has shown that both midwater and benthic species can be reliably depleted in this manner (Forney 1957). Data of this type yield very precise estimates of absolute abundance (Zippin 1958), and coefficients of variation associated with our abundance estimates were generally well under 5%. Therefore, we assumed that these small errors in abundance estimation would have a negligible contribution to error in estimation of capture efficiency.

Capture efficiencies estimated from removal sampling ranged from <10 to nearly 100%, with the majority of estimates >50% (Table 4). Mean efficiencies for midwater fish, 75% (density), and 83% (biomass), were similar to means for total fish, 72% (density), and 81% (biomass), reflecting the dominance of midwater fish at most of our sampling stations. Benthic fish capture efficiencies, which averaged 50% (density) and 68% (biomass), were considerably lower than values for midwater fish. Earlier studies have also shown roughly 20% differences between midwater and benthic fish capture efficiencies (Forney 1957; Lyons 1986; Parsley et al. 1989).

Capture efficiencies estimated from recaptures of marked fish ranged from 35 to 100%, and averaged 80% compared with 75% for removal estimates for density of midwater fish. However, correlation between these estimates was weak (Fig. 1), suggesting that simple efficiency estimates from recaptures of relatively few marked fish do not closely reflect true capture efficiency of the resident fish populations. Better estimates could probably be obtained from recaptures if larger numbers of marked fish were used, and the ambient mixture of species was more closely matched.

Predicting Capture Efficiency

Habitat and fish community variables explained a substantial portion of the variation in capture efficiencies (Table 5). Capture efficiency for sampling total fish density was a negative function of snags and the proportion of benthic fish, with model 1 in Table 5 explaining 52% of the variation in efficiency (Fig. 2a). Model 3 (Table 5) explained 73% of the variation in efficiency of sampling total fish biomass (Fig. 2c) as a positive function of macrophyte biomass, and a negative function of snags and seine rolling. Models 2 and 4 (Table 5) employ similar combinations of variables to explain 26 and 68% of the variation in capture efficiency for midwater fish density and biomass, respectively (Figs. 2b, d). No statistically significant model for benthic fish density was found, but model 5 (Table 5) explained 71% of the variation in efficiency for sampling benthic fish biomass as a positive function of mean benthic fish mass, and a negative function of seine rolling (Fig. 2e).

Frequency of snags and degree of seine rolling were important predictors of capture efficiency, and both had the expected negative effects in all cases. The proportion of benthic fish also had a negative effect on capture efficiency for sampling total fish density. Benthic fish are apparently more likely to escape underneath the bottom line of the seine than midwater species (Table 4; Forney 1957; Lyons 1986; Parsley et al. 1989). The positive effects of macrophyte biomass seen in Table 5 may at first seem counterintuitive, but are consistent with our observations during seining. Fish entrapped in the advancing seine seemed less agitated where macrophyte growth was extensive,

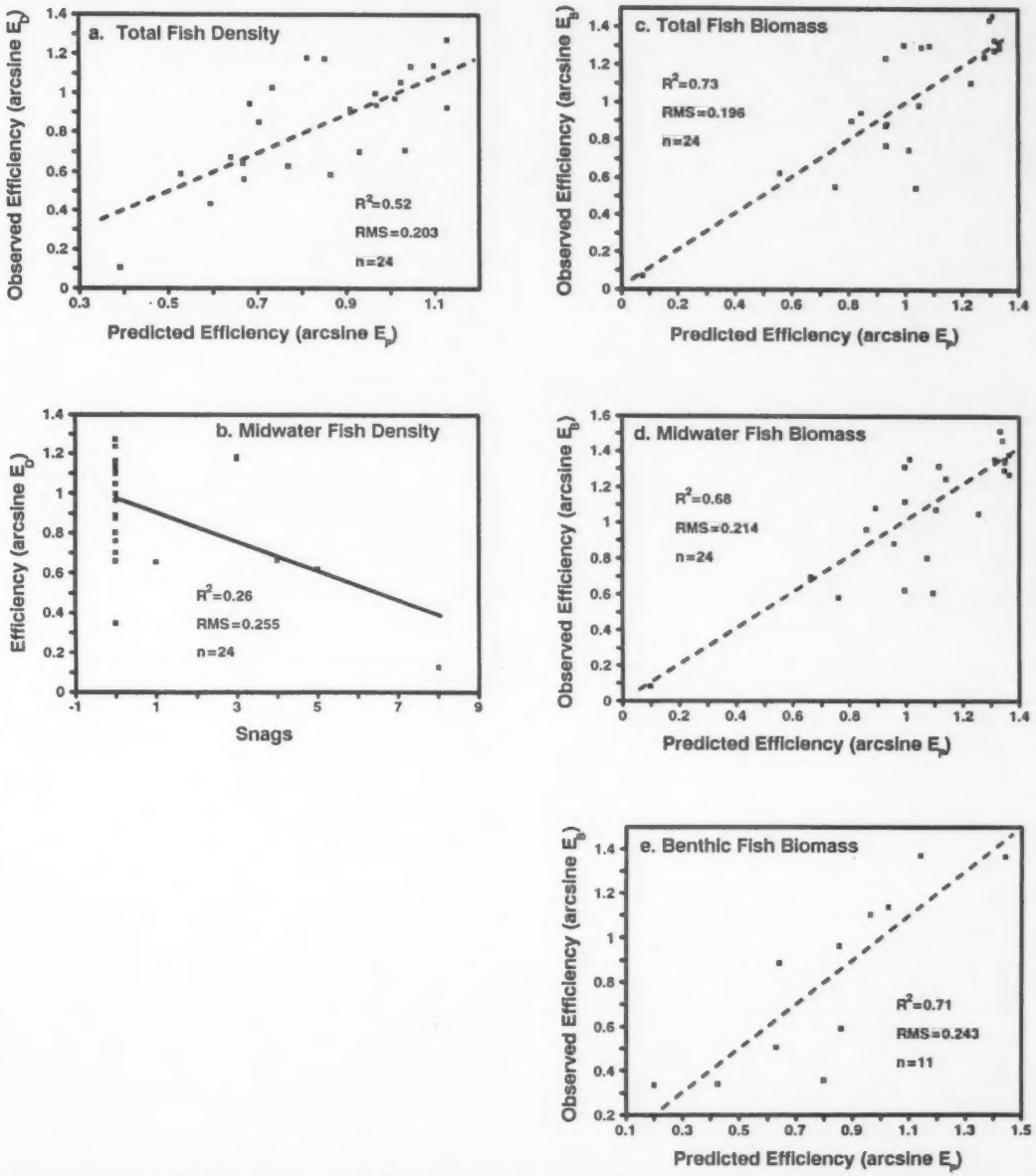

FIG. 2. Observed capture efficiency estimates versus predicted capture efficiency of a beach seine for sampling total, midwater, and benthic fish. (Note: simple bivariate relationship of capture efficiency and number of snags shown in b). Relationships a through e described in Table 5 by models 1 through 5, respectively. Dashed lines are 1-to-1 lines; solid line is a least-squares regression line.

and thus may have been less likely to find escape routes. Fish size was a significant predictor of capture efficiency only for benthic fish; smaller benthic fish were apparently better able to escape underneath the seine than larger fish.

The models in Table 5 provide predictions of capture efficiency based on a few easily measured variables. Predictions from these models can be used as correction factors to convert seine catches to abundance estimates by solving equation (1) for A. Moreover, these predicted correction factors incorporate the variability in capture efficiency inherent in sampling different fish communities from different habitats. Seining can provide quantitative estimates of littoral zone fish abundance under most conditions encountered in temperate lakes, provided that appropriate corrections for variable capture efficiency are made. We recognize that some conditions, such as dense emergent vegetation, flooded stands of timber, and other extreme circumstances may still preclude the use of seines in favor of other methods (e.g. Bayley and Austen 1988). How-

ever, our results effectively extend the range of suitable conditions for seining and significantly reduce the uncertainties surrounding abundance comparisons among diverse sites. These improvements can be achieved with very little additional effort. Sampling can be accomplished by two workers if necessary, but it is helpful to have an additional person in a boat freeing the seine from snags while the other two pull it toward shore from the ends. It is useful to have a diver for sampling macrophytes, but this could also be done from a boat with a remote grab or using an echosounder (Duarte 1987).

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Urchin Foraging and Algal Survival Strategies in Intensely Grazed Communities in Eastern Canada

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We examine relationships between food preferences of the urchin, *Strongylocentrotus droebachiensis*, and such properties of fleshy macroalgae as dry mass, ash content, calorific value, and phenolic content. Grazing resistant algae characteristic of urchin dominated habitats rank low in attraction, reflecting the ability of urchins to detect and locate them in the field. They fall into two groups, (1) reds, *Phycodrys rubens, Turnerella pennyi*, and *Ptilota serrata*, with a high proportion of dry matter and low phenolic content, and (2) browns, *Desmarestia viridis* and *Agarum cribrosum* with low to intermediate calorific values. Algae susceptible to grazing fall into two additional groups, (3) algae with high dry mass which strongly attract urchins and are consumed at an intermediate rate (mostly annuals), and (4) readily eaten perennials which attract urchins to an intermediate degree and have high ash and low phenolic content. The susceptible algae survive through spatial and temporal escapes, mainly in shallow water where the effectiveness of urchin grazing is reduced. The urchin is a selective feeder and its ability to locate preferred algae promotes intake of foods (algae in the subtidal algal fringe and drift algae) that enhance fitness by favouring somatic and gonadal growth.

Les auteurs ont étudié les rapports entre les préférences alimentaires de l'oursin, *Strongylocentrotus droebachiensis*, et certaines caractéristiques des macro-algues charnues telles que la masse sèche, la teneur en cendre, la valeur calorifique et la teneur en composés phénoliques. Les algues résistant au pâturage caractéristique des habitats où domine l'oursin présentent peu d'attrait, ce qui reflète la capacité des oursins à les détecter et à les localiser sur le terrain. Elles appartiennent à deux groupes (1) algues rouges, *Phycodrys rubens, Turnerella pennyi* et *Ptilota serrata*, qui présentent une forte proportion de matière sèche et une faible teneur en composés phénoliques et (2) les algues brunes *Desmarestia viridis* et *Agarum cribrosum* qui ont des valeurs calorifiques de faibles à intermédiaires. Les algues qui se prêtent au pâturage appartiennent à deux groupes supplémentaires (3) les algues à forte masse sèche qui attirent fortement les oursins et sont consommées à un rythme intermédiaire (en grande partie des annuelles) et (4) les perennes, facilement comestibles qui attirent les oursins à un degré intermédiaire et ont une forte teneur en cendre et une faible teneur en composés phénoliques. Les algues vulnérables survivent par la fuite dans l'espace et dans le temps, en grande partie dans les eaux peu profondes où l'efficacité du pâturage par les oursins est réduite. L'oursin est un prédateur sélectif et sa capacité à localiser ses algues préférées favorise l'absorption des aliments (les algues à la limite algale subtidale et les algues dérivantes) qui sont bons pour la santé et favorise la croissance somatique et gonadique.

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Strong interactions between herbivores and macrophytes in marine communities have stimulated a profusion of studies aimed at elucidating both the feeding strategies of the herbivores and the defensive strategies of the macrophytes (Lawrence 1975; Lubchenco and Gaines 1981; Hawkins and Hartnoll 1983; Vadas 1985). A common hypothesis is that predators (herbivores and carnivores) select food so as to maximize the intake of prey that enhance somatic and gonadal growth and thus increase their fitness (Emlen 1966; MacArthur and Pianka 1966; Schoener 1971; Pyke et al. 1977). In natural habitats, the diversity, availability, energetic value, and ease of consumption of potential prey vary markedly in space and time and could affect the evolution of feeding behaviours. For example, from theoretical considerations of the costs related to searching for prey, Emlen (1966) predicts that predators should feed indiscriminately when food is scarce, and show increasing selectivity as food abundance increases. Similarly, algae have probably evolved strategies to increase their fitness. In habitats perturbed by herbivores, plants may decrease predation by spatial or temporal avoidance of herbivores, or by defenses, such as calcification, increased toughness, and the production of toxic substances (for example, phenolics) to limit grazing (Vadas 1977; Nicotri 1980; Littler and Littler 1980; Geiselman and McConnell 1981; Steneck and Watling 1982; Steinberg 1985, 1988; Targett et al. 1986; Johnson and Mann 1986).

Urchin grazing is often a major factor determining community structure in subtidal communities (Leighton 1966; Jones and Kain 1967; Foreman 1977; Pearse and Hines 1979; Chapman 1981; Nédélec 1982; Sammarco 1982; Hagen 1983; Himmelman et al. 1983a; South 1983; Verlaque 1984, 1987; Harrold and Reed 1985; Carpenter 1986). For example, in Nova Scotia between 1970 and 1980 a marked increase in populations of the green sea urchin, *Strongylocentrotus droebachiensis*, led

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to transformation of kelp beds into urchin–coralline algal communities (Mann 1977; Chapman 1981). Mass mortalities of urchins in 1981 to 1983 caused by disease (Miller and Colodey 1983; Scheibling and Stephenson 1984) resulted in a return to the kelp dominated system (Moore and Mill 1983; Miller 1985; Johnson and Mann 1988).

North of Nova Scotia, in the northern Gulf of St. Lawrence and Newfoundland, benthic communities appear to be stable, with no apparent large-scale changes in urchin or algal populations (Himmelman 1969, 1985, 1986; Hooper 1980; Himmelman et al. 1983a,b; Keats et al. 1984a,b, 1985; Himmelman and Lavergne 1985). Due to ice abrasion or fresh water runoff, there may be localized mortalities of urchins and increases in algae. Small urchins are usually abundant so that reductions in grazing caused by periodic losses of larger urchins (the major macroalgal grazers) are rapidly compensated for by the growth of juveniles (Himmelman 1986). Similar communities where algal abundance is strongly limited by *S. droebachiensis* are found in northern Norway (Hagen 1983), the White Sea (Propp 1977), and Maine (R. L. Vadas, University of Maine, Orono, pers. comm.). In such communities algal survival is probably due to morphological or chemical defenses, to escapes related to physical factors, or to life cycle adaptations.

In the present study, we examine urchin-macroalgal interactions in the persistent urchin dominated communities of the northern Gulf of St. Lawrence. Specifically we: (1) describe the distribution of macroalgae and urchins and determine to what extent various algae resist urchin grazing; (2) quantify urchin food preferences; (3) examine how urchin food preferences are related to such algal properties as dry mass, ash content, calorific value, and phenolic content; (4) analyze patterns of algal defenses against urchin grazing; and (5) consider strategies of algae and of the urchin for optimizing fitness. In the northwestern Atlantic region, previous studies have focused on factors affecting urchin populations and their feeding activities. The present study is the first to elucidate the defensive strategies of the major fleshy macrophytes against the intensive urchin grazing of this region.

Methods

Study Site

The study was conducted at Cap du Corbeau, Ile du Havre, in the Mingan Islands (near Havre-Saint-Pierre), northern Gulf of St. Lawrence, eastern Canada (Fig. 1). This is a moderately exposed site with a gradient in substratum from bedrock and boulders in shallow water to sand and mud at greater depths.

Distribution of Algae and Urchins

Six vertical transects, extending from about 2 m above to 20 m below lowest water of spring tides (LWST), were placed at 100-m intervals along the perimeter of Cap du Corbeau (Fig. 1). The slope decreased from the north to the south side of the cape. The northernmost transect was 48 m long whereas the southernmost transect was 150 m long. In mid-August to early September, two divers examined 1×2 m quadrats placed at 1-m intervals along both sides of the six vertical transects. For each quadrat, they visually estimated the percentage of the surface covered by urchins and algae as well as the percentage cover of different substrata. The substratum categories were mud/sand (<0.02 cm), pebbles (0.02–6.4 cm), cobbles (6.4–25.6 cm), boulders (>25.6 cm), and bedrock. Prior to this sampling, practice sampling was made along three of the transects to ensure that estimates of percentage cover were similar (within 15%) for the two divers.

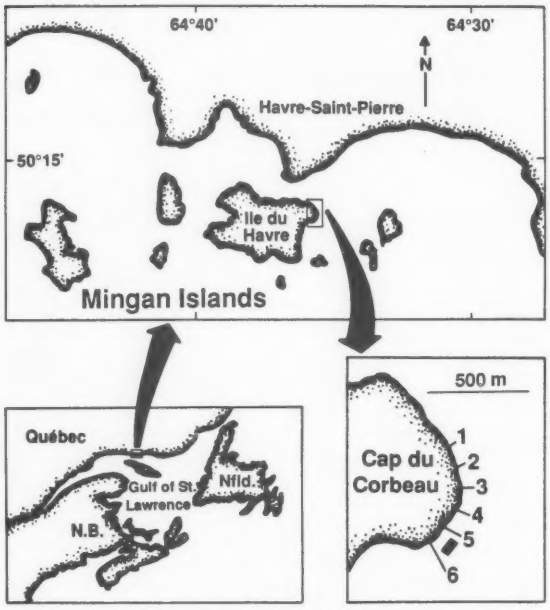

FIG. 1. Location of the study site at Cap du Corbeau, Gulf of St. Lawrence, and the relative position of the six transects. The site where we quantified the attraction of urchins for the various algae was between transects 5 and 6.

Urchin Attraction to Algae

We define attraction as the capacity of urchins to detect and locate their algal foods under field conditions and consider it to be a determinant of food preference. Water-borne substances from the algae probably play a major role in attraction. This is suggested by the upstream movement of *S. droebachiensis* towards algae observed in laboratory studies (Vadas 1977; Larson et al. 1980; Mann et al. 1984). For example, Mann et al. (1984) show that during the spring and summer 70–79% of urchins placed in a choice chamber move in the direction of algae or algal extracts within 5 min. The relative levels of attraction were quantified for 18 species of algae in a flat and homogeneous site at 10 m deep (south side of Cap du Corbeau; Fig. 1). The substratum was mainly pebbles with encrusting coralline algae, *Lithothamnium* spp. and *Clathromorphum* spp., and the density and size structure of the urchin population were remarkably uniform throughout this site. A few fleshy macrophytes (*Desmarestia viridis* and *Agarum cribosum*) present in the experimental area were removed by hand prior to the experiment. A grid pattern was set up by placing steel spikes in horizontal and vertical lines, with 3-m distances between spikes. To measure attraction, 60-g monospecific bundles of 18 species of algae (held together with wire) were attached at random to the different spikes and after a 24-h experimental period the number of urchins observed within a 0.25-m^2 quadrat around each algal bundle was counted. Only urchins visible at the surface of the substratum were counted (see Results for a consideration of this potential bias). Turning the rocks to search for

hidden urchins would have been laborious and would have disrupted the study site. In preliminary trials with bundles of *Laminaria digitata* and *Agarum cribrosum* weighing 7.5, 15, 30, 60, 120, and 240 g (three replicates of each weight for each alga), the number of urchins tended to increase as the bundle size increased from 7.5 to 30 g and then to stabilize; however, a Spearman's test showed no significant effect of bundle weight on the number of urchins attracted ($r_s = 0.32$ for *Laminaria*, $n = 18$; and $r_s = -0.02$ for *Agarum*, $n = 18$). Between mid to late June 1985, 10 measures of attraction were obtained for each alga. One or two days were left between each experimental run to allow the urchins to redistribute themselves throughout the experimental site in the absence of fleshy algae. During each run, two randomly chosen spikes were left without algae to provide control values of urchin density. The ambient temperature during the experimental period was 5–7°C.

Urchin Feeding Rates

Feeding rate, involving both tactile responses and the ability to manipulate various foods, is another determinant of food preference. The rates at which urchins ingest the various algae were determined in July at an ambient temperature of 4–8°C. We measured the wet algal mass consumed per day by groups of four urchins (40–45 mm in diameter) provided with single algal diets in 4-L pails (20 cm in diameter) supplied with running seawater. A mean feeding rate (g wet mass eaten·urchin^{-1}·d^{-1}) was obtained from 10 determinations for each alga studied.

Algal Characteristics

Preweighed (wet mass) samples of each of the 18 algae used in the attraction experiments were frozen for later determinations of dry mass, ash content, calorific value and phenolic content. Percentage dry mass was recorded after a drying period of 48 h at 45°C, ash content per gram dry mass was measured after burning the samples at 450°C for 16 h. The calorific values of algae (Kcal·g ash free dry mass; 1 calorie = 4.184 joules), were determined by combustion in a Parr microbomb calorimeter (model 1341). Total phenolic content was determined using the Folin-Ciocalteau technique (Singleton and Rossi 1965). Phenolics were extracted from 2-g samples of algae (fresh weight) using 70% methanol at 8°C for 24 h. The absorption was read at 725 nm (Bausch and Lamb Spectronic 2000) with gallic acid as the reference compound. For each alga, we obtained measurements of the above characteristics from 10 specimens, except for ash content of *Laminaria digitata* and *Scytosiphon lomentaria* for which we obtained measurements for nine and four specimens, respectively.

Statistical Analyses

To compare distribution patterns among subtidal macrophytes at Cap du Corbeau, they were classified based on their co-occurrence in the 932 quadrats using the Fager and McGowan (1963) similarity index. Clustering of the algae was obtained using the flexible grouping method ($\beta = 0.25$). The values for consumption, attraction, and all of the algal characteristics, except calorific content, were normally distributed ($P < 0.01$; $N = 173$–180). Thus a parametric analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to examine interalgal variations in these parameters. The Kruskal-Wallis ANOVA was used to test for interalgal differences in calorific content, since the calorific values were not normally distributed (Kolmogorov-Smirnov test; $P = 0.079$) and could not be normalized. To evaluate different trends in the characteristics of the various algae, we performed a principal component analysis using a correlation matrix for all the variables (Cooley and Lohnes 1971).

Results

Macroalgal and Urchin Distributions

Algae formed a dense zone in shallow water but showed a drop in abundance generally near lowest water of spring tides (LWST), where urchins became abundant (Fig. 2). In the shallowest part of transect 6 on the exposed side of Cap du Corbeau, urchins were few and there was a dense algal bed dominated by *Alaria esculenta*. By contrast, urchins were abundant near low water level in transects 1, 2, and 3 on the protected side of Cap du Corbeau and algal cover decreased at shallower depths. Exposure to waves was similar in transects 4 and 5, but macroalgae penetrated to greater depths in transect 4 where the bottom was more gently sloped. Most urchins in the shallow water bedrock zone bordering the algal fringe measured 28–55 mm in diameter, whereas at greater depths and distance from the algal fringe most urchins measured <30 mm in diameter and two modes were evident, one made up of 5–8-mm individuals and a second of larger individuals (Fig. 3).

The various algae showed striking differences in vertical distributions (Table 1). Twelve were found only in shallow water (to 1 or 2 m), whereas seven other algae were present throughout the subtidal zone. Cluster analysis performed on the presence and absence of algae in the 932 quadrats, similarly showed the presence of two distinct groups and further indicated five subgroups of algae (Fig. 4). The first three groups were from shallow water and consisted of (1) *Fucus* spp. which formed a distinct band terminating in the low intertidal zone, (2) *Ulvaria obscura, Spongomorpha arcta, Devaleraea ramentacea, Porphyra miniata, Dictyosiphon foeniculatus, Alaria esculenta, Chordaria flagelliformis, Saccorhiza dermatodea,* and *Laminaria longicruris,* which were the principal macroalgae of the fringe zone, and (3) *Laminaria digitata* and *Rhodomela confervoides*, which were also algae of the fringe zone but were more localized in their distributions. Several of the algae of the second group were responsible for a characteristic zonation pattern near low water level, first a green zone of *Spongomorpha,* followed by a red zone of *Devaleraea* and finally a brown zone of *Alaria*. The remaining two groups were from the urchin dominated zone and consisted of (4) *Agarum cribrosum, Antithamnion* sp., *Turnerella pennyi, Desmarestia viridis,* and *Callophyllis cristata*, and (5) *Ptilota serrata* and *Phycodrys rubens*.

Within the rocky zone dominated by urchins, we often noted that *Agarum, Antithamnion, Turnerella, Desmarestia, Callophyllis, Ptilota,* and *Phycodrys* were most common on the sides and tops of boulders. This association was corroborated by the quantitative analysis (Table 2). The frequency of occurrence of boulders was significantly greater in quadrats where these algae were present than throughout the urchin dominated rocky zone (2–14 m deep; thus from just below the algal fringe to where rocky substrata were rare). Conversely, the occurrence of pebbles was lower in quadrats where these algae were present than in the overall urchin dominated rocky zone.

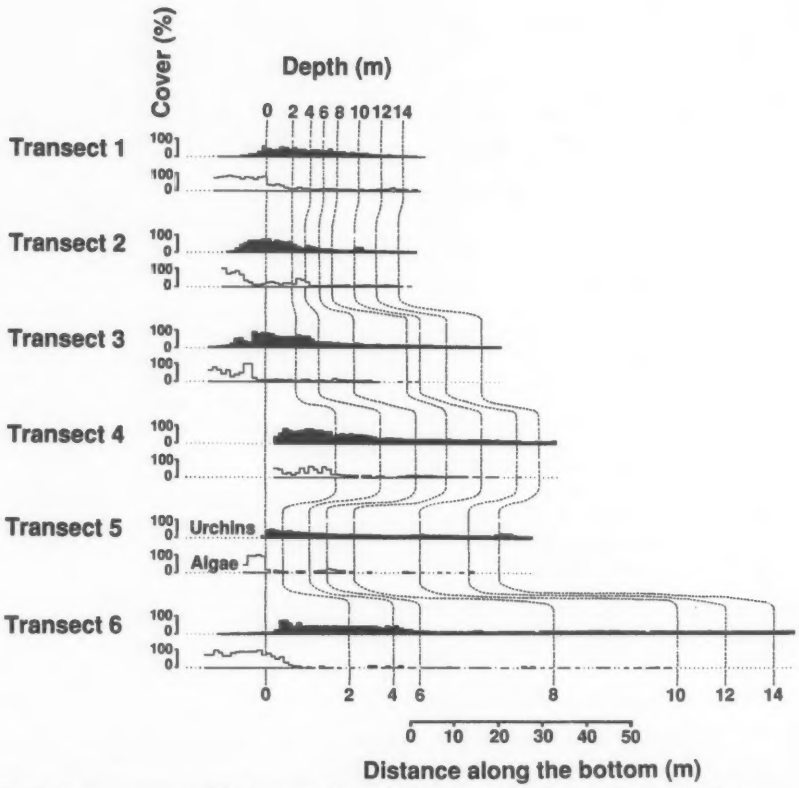

FIG. 2. Percentage cover of fleshy macroalgae and urchins at 1-m depth intervals along the six transects at Cap du Corbeau from 2 m above LWST, in low intertidal zone, to a maximum depth of 14 m below LWST.

Urchin Food Preferences

Attraction

In studying attraction, we considered only the urchins which were visible at the surface of the substratum. Most juveniles (<15 mm in diameter) were hidden and were not a part of our estimates of attraction (Fig. 5A, B). Even in the presence of the most attractive algal foods, these juveniles did not move towards macroalgae, thus indicating a sedentary behaviour (Fig. 5A and B compared to D). Our experimental design permitted us to measure directly the attraction of urchins towards the 18 fleshy macroalgae. The number of urchins in the control samples taken at sites where no algae were placed (23.3, SE = 6.55, $N = 10$), was not significantly different (t-test, $P > 0.05$) from the number of urchins in the experimental area prior to the experiment (28.0, SE = 5.09, $N = 12$). Thus, the 3-m distance between spikes to which the algae were attached was apparently sufficient to prevent the most attractive algae from depleting the supply of urchins in the vicinity of adjacent spikes.

Some algae attracted far more urchins than others (ANOVA, $F = 16.47$, $P < 0.001$; Fig. 6). A classification using a Duncan's multiple range test (DMRT; Table 3) separated the algae into four homogeneous groups, (1) *Chordaria, Ulvaria,* and *Alaria,* which were extremely attractive, (2) *Fucus, Scytosiphon,* and *Laminaria digitata* with intermediate attractiveness, (3) *Spongomorpha, Rhodomela,* and *Saccorhiza* which were only slightly attractive, and (4) *Palmaria, Porphyra, Desmarestia, Agarum, Phycodrys, Turnerella, Devaleraea,* and *Ptilota* which were non-attractive. *Ascophyllum* was not separated from either the slightly attractive or the non-attractive algae. With the exception of *Rhodomela*, the species which significantly attracted urchins were phaeophytes or chlorophytes, whereas the non-attractive algae were phaeophytes, previously reported to be resistant to grazing (*Agarum, Desmarestia;* Vadas 1977; Himmelman 1985), or rhodophytes.

Urchins in the vicinity of the five algae with the highest attraction values (*Chordaria, Ulvaria, Alaria, Fucus,* and *Scytosiphon*) were significantly larger than urchins collected in the control sites (χ^2, $P < 0.05$; Fig. 5). This is probably attributable to an increased mobility of larger urchins. By contrast, urchins collected at the *Turnerella* sites were smaller than at control sites ($P < 0.05$; Fig. 5E), indicating that urchins may avoid *Turnerella.*

Feeding Rate

Strong differences were also evident in the rates at which urchins consumed the various algae (ANOVA, $F = 32.64$, $P < 0.001$; Table 4). A DMRT separated the algae into four relatively homogeneous groups: (1) *Rhodomela, Palmaria,*

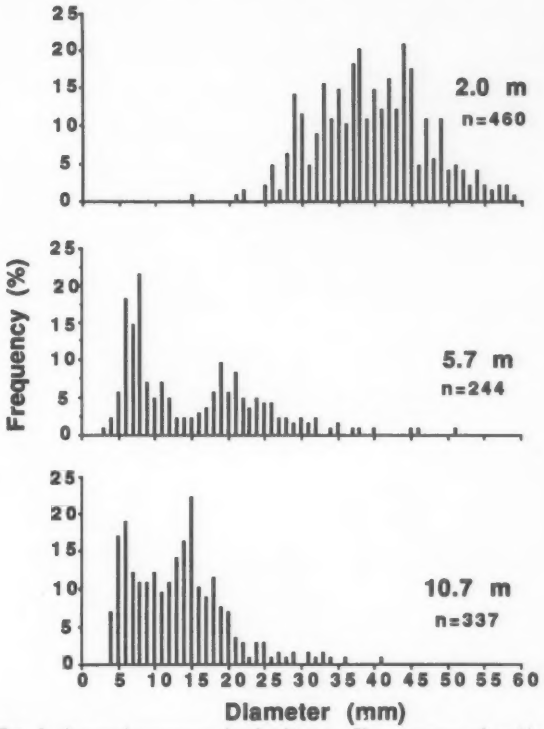

FIG. 3. *Strongylocentrotus droebachiensis*. Size structure of urchin populations at 2 m in depth, on bedrock zone just below the algal fringe, at 5.7 m where the substratum was mainly boulders (45% cover) and cobbles (36%), and at 10.7 m in depth, where pebbles predominated (85%). The sampling was conducted in the vicinity of transects 5 and 6.

Spongomorpha, *Saccorhiza*, and *Devaleraea* which were rapidly consumed (1.09–1.22 $g \cdot d^{-1}$); (2) *Fucus*, *Chordaria*, and *Scytosiphon* which were ingested at a slower rate; (3) *Ulvaria*, *Ascophyllum*, *Alaria*, *Porphyra*, *Desmarestia*, and *Agarum* which were ingested at a significantly lower rate than all of the above algae (0.34–0.90 $g \cdot d^{-1}$), and (4) *Ptilota*, *Turnerella*, and *Phycodrys* which were eaten at a very low rate (0.06–0.21 $g \cdot d^{-1}$). The latter five algae were characteristic of the urchin barrens and must be considered resistant to urchin grazing. The low rate at which they are eaten by urchins probably contributes to survival under intensive grazing. *Laminaria digitata* was intermediate between the first two groups. (Urchins ingested *Laminaria longicruris* at a rate of 0.74 $g \cdot urchin^{-1} \cdot d^{-1}$, SE = 0.064; it would be placed in the second group in a DMRT analysis). There was little relation between feeding rate and phylogenetic classification, except that the least consumed species were red algae. Moreover, there was little relation with morphology, except that the three resistant red algae were generally small (<15 cm in height).

Comparison of Attraction and Feeding Rate

For the 18 algae considered together, attraction was not correlated with feeding rate ($r_s = 0.43$; $P = 0.07$). Attraction and feeding rate were both low for *Ascophyllum* and the algae found on urchin barrens (*Turnerella*, *Phycodrys*, *Ptilota*, *Agarum*, *Desmarestia*, and *Porphyra*), however, they were significantly negatively correlated for the remaining 11 algae ($r_s = -0.78$; $P < 0.01$). As a result a number of algae were grouped very differently (DMRT) using these two preference measures (Tables 3 and 4). The algae which most strongly attracted urchins, *Chordaria*, *Ulvaria*, and *Alaria*, were only eaten at intermediate rates, whereas the algae which were most rapidly eaten, *Palmaria*, *Rhodomela*, *Devaleraea*, *Spongomorpha*, and *Saccorhiza*, ranked only intermediate to low in attraction (Tables 3 and 4). Generally, urchins were weakly attracted to red algae, irrespective of the rate at which they were eaten.

Relationships Between Algal Properties and Urchin Food Preferences

Mean values of percentage dry mass, ash content, and phenolic content differed among the 18 algae (parametric ANOVA), as did calorific value (Kruskal-Wallis ANOVA), (probability of equal means <0.001 for all of the above parameters).

Red algae, except for *Palmaria* and *Devaleraea*, had higher percentage dry mass than brown algae, whereas the two green algae, *Ulvaria* and *Spongomorpha*, had high and low values, respectively (Table 5). A Spearman's correlation analysis using the 18 algae indicated that attraction was not significantly correlated with dry mass ($r_s = -0.20$, $P > 0.05$; Fig. 7). By contrast, feeding rate was negatively correlated with dry mass ($r_s = -0.52$, $P < 0.05$; Fig. 7).

Attraction was not significantly correlated with either ash or organic matter content. By contrast, feeding rate was positively correlated with ash content ($r_s = 0.53$, $P < 0.05$; Fig. 7) and negatively correlated with percentage organic matter (the inverse of ash content). Ash content per gram dry mass was strongly negatively correlated with dry mass ($r_s = -0.75$, $P < 0.001$) and organic matter content showed the opposite relationship. For all of the algae from the urchin dominated zone (*Agarum*, *Desmarestia*, *Phycodrys*, *Ptilota*, and *Turnerella*), ash content per gram dry mass was low (20.9–25.5%; Fig. 7; Table 5) and organic matter content high (74.5–79.1%).

The brown, red, and green algae all showed marked variations in energy content. Neither attraction ($r_s = -0.12$, $P > 0.05$) nor feeding rate ($r_s = -0.02$, $P > 0.05$) was correlated with calorific value per gram ash free dry mass (Table 5; Fig. 7). For example, the algae from the urchin zone varied from lowest (*Desmarestia*) to highest (*Ptilota*) calorific values. The same results were obtained using calorific value per gram dry mass and calorific value per gram wet mass.

The phenolic content per gram wet mass of brown algae was strikingly higher than that of red algae, except for *Rhodomela*. The green algae *Ulvaria* and *Spongomorpha* had high and low phenolic contents, respectively. The brown alga, *Agarum*, had the highest phenolic content, whereas another brown alga, *Desmarestia*, had a low phenolic content. Attraction was positively correlated with phenolic content ($g \cdot wet\ mass^{-1}$) for the 18 algae treated together ($r_s = 0.53$, $P < 0.05$), but not for browns treated separately ($r_s = 0.09$, $n = 9$, $P > 0.05$). Feeding rate was not significantly correlated with phenolic content, either for all of the algae treated together, or for brown algae treated separately (Table 5; Fig. 7). The same results were obtained when phenolic content was expressed in grams dry mass.

Both calorific intake and organic matter intake were strongly positively correlated with feeding rate ($r_s = 0.95$, $P < 0.001$ and $r_s = 0.96$, $P < 0.001$, respectively). This was expected due to the marked effect of feeding rate on these intake values. However, attraction was also positively correlated with both calor-

TABLE 1. Percentage cover (and percentage occurrence) of various types of substratum, of fleshy macroalgae and of urchins at 2-m depth intervals along the six transects at Cap du Corbeau. Lowest water of spring tides is taken as 0 depth.

	Water depth (m)									
	2–0	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18
Number of quadrats	102	118	76	64	122	128	92	82	72	66
Substratum										
Bedrock	32.1 (35)	40.1 (46)	5.7 (11)	4.1 (6)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Boulders	58.2 (75)	49.3 (66)	66.6 (96)	52.4 (95)	27.4 (74)	12.6 (64)	5.4 (47)	5.3 (24)	.7 (14)	.0 (2)
Cobbles	8.3 (47)	8.6 (51)	20.5 (78)	27.9 (81)	17.9 (77)	14.8 (76)	13.1 (72)	6.0 (45)	3.0 (31)	.4 (15)
Pebbles	1.4 (22)	2.0 (25)	6.2 (62)	14.2 (63)	47.0 (88)	48.4 (92)	38.5 (95)	32.0 (74)	27.5 (92)	26.0 (100)
Sand and mud	.0 (1)	.1 (7)	1.0 (28)	1.4 (61)	7.7 (88)	24.2 (96)	43.1 (97)	56.7 (100)	78.8 (100)	73.7 (100)
Algae										
Fucus spp.	1.7 (22)	.1 (4)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Dictyosiphon foeniculatus	.3 (21)	.0 (1)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Rhodomela confervoides	.2 (13)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Spongomorpha arcta	4.0 (44)	.2 (4)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Devaleraea ramentacea	9.5 (47)	.1 (4)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Porphyra spp.	1.6 (37)	.1 (1)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Saccorhiza dermatodea	1.9 (28)	.8 (4)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Laminaria longicruris	.3 (13)	.4 (3)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Laminaria digitata	7.8 (26)	2.0 (8)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Alaria esculenta	20.0 (74)	4.8 (38)	.0 (1)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Chordaria flageliformis	6.0 (51)	.1 (6)	.2 (4)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Ulvaria obscura	8.2 (69)	.3 (15)	.0 (1)	.0 (5)	.0 (2)	.0 (0)	.0 (0)	.0 (0)	.0 (0)	.0 (0)
Desmarestia viridis	.0 (1)	.5 (6)	.2 (8)	.3 (5)	.0 (3)	.0 (2)	.0 (4)	.1 (6)	.0 (0)	.0 (0)
Agarum cribrosum	6.2 (44)	8.5 (54)	3.8 (39)	1.6 (17)	.2 (12)	.1 (11)	.1 (8)	.2 (11)	.0 (3)	.0 (3)
Antithamnion sp.	.1 (7)	.5 (36)	.6 (37)	.8 (44)	.8 (30)	.2 (13)	.4 (17)	.8 (22)	.0 (4)	.0 (0)
Ptilota serrata	.1 (5)	.2 (5)	.0 (1)	.0 (2)	.0 (1)	.0 (1)	.0 (1)	.0 (2)	.0 (1)	.0 (0)
Phycodrys rubens	.0 (4)	.2 (9)	.0 (3)	.0 (2)	.0 (3)	.0 (3)	.0 (1)	.0 (5)	.0 (1)	.0 (2)
Callophyllis cristata	.0 (1)	.1 (5)	.0 (0)	.0 (0)	.0 (0)	.0 (4)	.1 (11)	.3 (17)	.1 (8)	.0 (0)
Turnerella pennyi	.0 (0)	.1 (10)	.2 (14)	.5 (44)	.5 (39)	.3 (32)	.1 (14)	.0 (4)	.0 (4)	.1 (9)
Urchin										
Strongylocentrotus droebachiensis	16.1 (73)	52.2 (100)	41.2 (100)	25.9 (100)	12.6 (100)	10.8 (100)	11.3 (100)	7.7 (100)	6.7 (100)	3.6 (100)

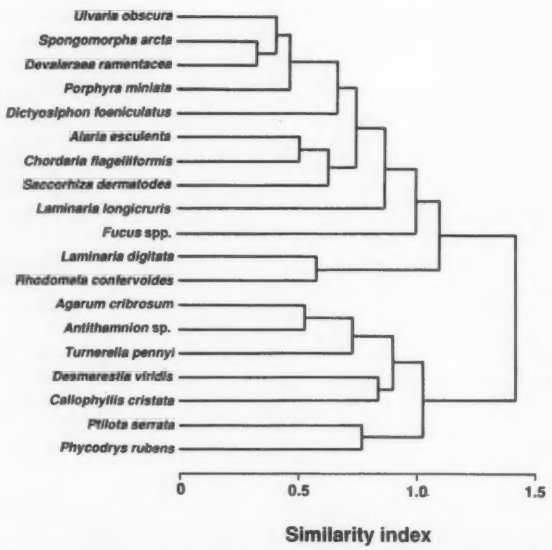

FIG. 4. Cluster analysis of algal co-occurrence, based on the 932 quadrats from the six transects at Cap du Corbeau.

TABLE 2. Percentage occurrence of boulders, cobbles, or pebbles in quadrats where grazing resistant algae were present, based on 564 quadrats examined between 2 and 14 m below lowest water of spring tides (the rocky subtidal zone below the subtidal algal fringe).

Algae (number of quadrats where the alga was present)	Percent occurrence of boulders	Percent occurrence of cobbles	Percent occurrence of pebbles
Agarum cribrosum (86)	94.2[a]	68.6	58.1[b]
Desmarestia viridis (25)	100.0[a]	64.0	52.0[b]
Turnerella pennyi (143)	93.7[a]	70.6	72.7
Phycodrys rubens (16)	100.0[a]	62.5[b]	50.0[b]
Ptilota serrata (7)	85.7[a]	71.4	28.6[b]
Callophyllis cristata (29)	89.7[a]	82.8[a]	69.0[b]
Antithamnion sp. (144)	97.2[a]	77.1	68.8[b]
Entire zone (564)	65.4	71.8	84.4

[a]Significantly greater than the overall percent occurrence of that substrate between 2 and 14 m deep.

[b]Significantly less than the overall percent occurrence of that substrate between 2 and 14 m deep.

ific intake ($r_s = 0.43$, $P < 0.05$) and organic matter intake ($r_s = 0.43$; $P < 0.05$). Phenolic intake was not correlated with feeding rate ($r_s = 0.29$; $P > 0.05$) but was positively correlated with attraction ($r_s = 0.63$; $P < 0.01$).

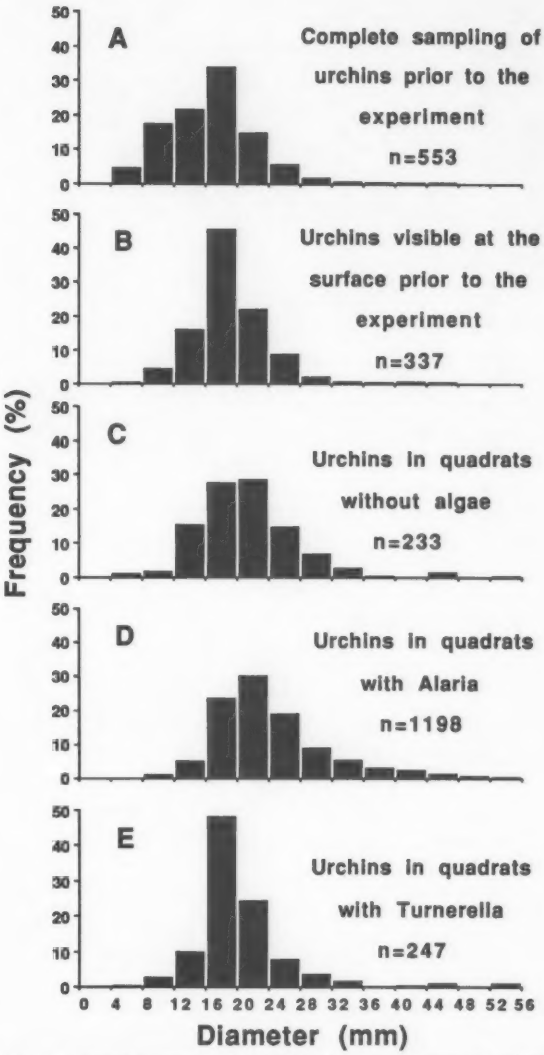

FIG. 5. *Strongylocentrotus droebachiensis*. Size structure of urchins in the experimental area prior to the experiment: (A) Complete sampling of 12 quadrats (50 × 50 cm), compared with (B) collections of urchins visible at the surface of the substratum (12 quadrats). Size structure of urchins during the experiment: (C) from 10 quadrats placed around spikes without algae, (D) from 10 quadrats placed around the preferred alga, *Alaria esculenta*, and (E) from 10 quadrats placed around the grazing resistant alga, *Turnerella pennyi*. (n = total number of urchins measured).

Trends in Characteristics Among Grazing Resistant and Preferred Algae

A principal component analysis was performed on mean values of the six algal parameters (attraction of urchins, feeding rate of urchins, percentage dry mass, ash content per gram dry mass, calorific value per gram ash free dry mass, and phenolic content per gram wet mass) for the 18 algae studied (Fig. 8). These parameters were weakly correlated, except for dry mass and ash content which were inversely correlated (Pearson's $r = -0.75$, $P < 0.001$). The first three axes accounted for 81% of total variance (38, 25, and 18% for axes 1, 2, and 3, respectively). Axis 1 was defined mainly by dry mass, axis 2 by phenolic content, and axis 3 by attraction. Axis 4, which only accounted for 10% of the variance, was defined by both phenolic content and calorific value. Five trends were evident among the 18 algae based on their proximity in four-dimensional space (the first four axes of the analysis): (1) *Palmaria, Devaleraea, Laminaria,* and *Spongomorpha,* with high ash content per dry mass (and thus low organic matter content), high calorific content, high consumption, and low phenolic content; (2) *Scytosiphon, Rhodomela, Chordaria, Alaria,* and *Ulvaria,* with high attraction and high dry mass; (3) *Phycodrys, Turnerella, Porphyra,* and *Ptilota,* with high dry mass, low consumption, and low phenolic content; (4) *Ascophyllum, Agarum, Fucus,* and *Desmarestia,* with high phenolic content and low calorific value; and (5) *Saccorhiza* with highest ash content per dry mass (lowest organic matter content), lowest dry mass and lowest calorific value. The high consumption and low phenolic content of *Saccorhiza* indicated a similarity with the first group. Thus, the resistant algae, which were eaten at a low rate and weakly attracted urchins, were separated into groups 3 and 4 by phylogenetic position (reds and browns, respectively). Of the preferred algae, the most attractive species, group 2, had a high dry mass and organic matter content and were only eaten at an intermediate rate. By contrast, the most readily eaten algae, group 1 (and 5) had low dry mass, organic matter and phenolic content, and attracted urchins at only an intermediate rate.

Discussion

Despite the intensive grazing pressure in subtidal communities in the northern Gulf of St. Lawrence, a number of fleshy macroalgae persist with urchins. We found that five of the most common species from the urchin zone, *Agarum cribrosum, Turnerella pennyi, Desmarestia viridis, Ptilota serrata,* and *Phycodrys rubens*, were clearly not preferred by urchins. These resistant algae did not attract urchins and were eaten at a very slow rate even when no other algae were available.

Algal Characteristics Related to Grazing Resistance

Although the multivariate analysis indicated four major patterns among the 18 algae studied, no characteristics other than measures of urchin food preferences were exclusive to the grazing resistant algae (Fig. 8). This suggests that these algae have evolved different strategies to limit grazing. Resistant algae fall into two distinct groups, red algae, *Phycodrys, Turnerella,* and *Ptilota,* and brown algae, *Desmarestia* and *Agarum*.

The resistant red algae and *Porphyra* are eaten slowly and have high dry mass and low phenolic content. Their morphology does not appear to be a major defense against urchin grazing, because they are small and relatively soft in structure. An alternative explanation is that chemical substances account for resistance; however, such defenses remain to be demonstrated. Their low phenolic content suggests either that other substances deter grazing, or that the phenolics present are extremely potent. Anderson and Velimirov (1982) suggest that non-preferred red algae with low phenolic levels may contain halogenated compounds that are unattractive to urchins. Similarly, Fenical (1975) hypothesizes that halogenated substances deter grazing. However, Fenical's (1975) data for four of the algae we studied do

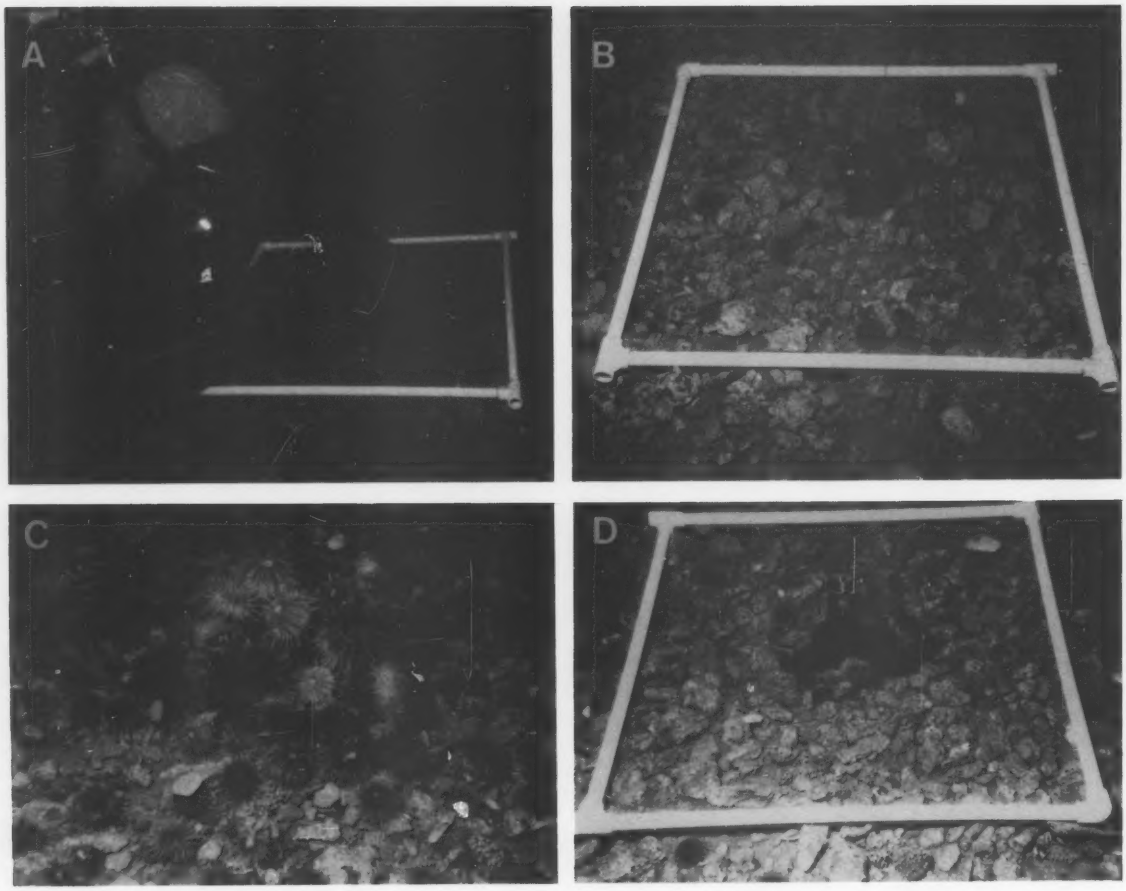

FIG. 6. (A) Diver sampling urchins in a site where 60 g of *Chordaria flagelliformis* had been attached to a spike 24 h earlier. A pile of urchins completely covers the alga and the spike. (B) A 0.25-m^2 quadrat placed over a spike where 60 g of *Ptilota serrata* had been attached 24 h previously. No urchins are in contact with the alga and the urchin density is similar to that at sites without algae. (C) A pile of urchins covering a spike where 60 g of *Alaria esculenta* had been attached 24 h previously. (D) A moderate number of urchins attracted to 60 g of *Agarum cribrosum*.

not support this hypothesis; thus, halogenated substances are present in *Phycodrys* (resistant) and *Rhodomela* (preferred) but absent in *Devaleraea* (preferred) and *Porphyra* (non-preferred). The defenses of these grazing resistant reds remain to be elucidated.

The resistant brown algae *Agarum* and *Desmarestia* are grouped with *Fucus* and *Ascophyllum*. These algae have intermediate to low calorific contents and, with the exception of *Desmarestia*, high phenolic contents. *Agarum* has the highest phenolic content of the 18 algae studied, indicating that phenolics could play a role in its resistance. Vadas (1977) and Larson et al. (1980) suggest, on the basis of low feeding rates, that chemical defences play a major role in protecting *Agarum* against grazing. Chemical studies are needed to test this hypothesis. Nevertheless, our data suggest that chemical defenses are less important for *Agarum* than for *Ptilota*, *Turnerella*, and *Phycodrys*. For example, the mean feeding rate of urchins on these algae was lower than that of urchins on *Agarum*, (DMRT, Table 4). Further, mean attraction for *Ptilota*, *Turnerella*, and *Phycodrys* tended to be lower relative to *Agarum* (Table 3). The resistant reds are small and sometimes form beds on low-lying rocky substrata, whereas *Agarum* appears more often to be restricted to boulders and vertical bedrock surfaces (J. H. Himmelman and H. Nédélec, pers. observ.). As its blade is kept in motion by wave action, attacks by urchins are limited (Himmelman 1985). This contributes to the survival of *Agarum* on elevated bedrock surfaces. In addition the stipe of *Agarum* is relatively tough, which probably confers grazing resistance.

Steinberg (1985, 1988) reports that phenolics of brown algae defer feeding by gastropod and echinoid herbivores on the Pacific coast of North America; however, our correlative data suggest that this is not the case in the urchin–algal interaction in eastern Canada. Thus, neither attraction nor consumption were inversely correlated with phenolic content for the nine brown algae in our study. In fact, phenolic content was positively correlated with attraction for the 18 algae studied ($r_s = 0.53$, $P < 0.05$).

Annual species of the genus *Desmarestia* are unusual because of their ability to produce sulphuric acid (Eppley and Bovell 1958; Thompson 1988) and the low rates of feeding and attrac-

TABLE 3. Mean attraction of urchins to each of the algal species. Attraction represented the number of urchins within a 0.25-m^2 quadrat placed around each algal sample after a 24-h experimental period (10 determinations were made for each alga). Means sharing a common letter were not significantly different (DMRT, $P>0.05$). The control value represents the number of urchins at sites (spikes) chosen at random where no algae were placed.

Species	Attraction (urchins·0.25 m^{-2}) (SE)	Duncan's multiple range test grouping
Chordaria flagelliformis	129.5 (14.22)	A
Ulvaria obscura	128.3 (16.69)	A
Alaria esculenta	119.8 (8.50)	A
Fucus edentatus	97.2 (12.17)	B
Scytosiphon lomentaria	83.1 (9.65)	B
Laminaria digitata	79.2 (13.15)	B
Spongomorpha arcta	64.6 (8.12)	C
Rhodomela confervoides	60.3 (6.97)	C
Saccorhiza dermatodea	60.2 (9.00)	C
Ascophyllum nodosum	39.8 (9.95)	C,D
Palmaria palmata	34.8 (4.28)	D
Porphyra miniata	34.3 (7.32)	D
Desmarestia viridis	29.4 (9.13)	D
Agarum cribrosum	28.3 (5.39)	D
Phycodrys rubens	26.7 (6.26)	D
Turnerella pennyi	24.7 (4.87)	D
Devaleraea ramentacea	24.5 (6.01)	D
Ptilota serrata	17.7 (5.06)	D
Control	23.3 (6.55)	D

TABLE 4. Mean consumption rate of 40–45-mm urchins on single algal diets. Each mean is based on 10 observations, and each observation represents the grams consumed by four urchins during a period of 24 h divided by 4. Means sharing a common letter were not significantly different (DMRT, $P>0.05$).

Algae	Feeding rate (g·urchin·d^{-1}) (SE)	Duncan's multiple range test grouping
Rhodomela confervoides	1.22 (0.085)	A
Palmaria palmata	1.21 (0.124)	A
Spongomorpha arcta	1.09 (0.057)	A
Saccorhiza dermatodea	1.07 (0.105)	A
Devaleraea ramentacea	1.05 (0.066)	A
Laminaria digitata	1.02 (0.084)	A,B
Fucus edentatus	0.90 (0.076)	B
Chordaria flagelliformis	0.88 (0.050)	B
Scytosiphon lomentaria	0.84 (0.060)	B
Ulvaria obscura	0.56 (0.036)	C
Ascophyllum nodosum	0.50 (0.065)	C
Alaria esculenta	0.48 (0.060)	C
Porphyra miniata	0.47 (0.048)	C
Desmarestia viridis	0.39 (0.079)	C
Agarum cribrosum	0.34 (0.037)	C
Ptilota serrata	0.21 (0.064)	D
Turnerella pennyi	0.06 (0.016)	D
Phycodrys rubens	0.06 (0.024)	D

tion for *D. viridis* may be related to this characteristic. *D. viridis* becomes pale in colour within 5–10 min of being removed from water, indicating a high rate of acid production. Low consumption of *D. viridis* was also observed by Himmelman (1985) although a higher rate of consumption was reported by Keats et al. (1983, 1984b). High sulphuric acid content may similarly explain the low palatability of *Desmarestia firma* for the urchin *Parechinus angulosus* (Anderson and Velimirov 1982). The delicate branched structure of *Desmarestia viridis* is an additional factor contributing to its resistance, because its movement with the slightest wave action limits attacks by urchins (Himmelman 1985).

Ascophyllum is an intertidal species not exposed to urchin predation but its characteristics, except for the high calorific content, resemble the grazing resistant browns, *Agarum* and *Desmarestia*. Geiselman and McConnell (1981) show that *Ascophyllum* produces polyphenolics which protect it from the common intertidal herbivore, *Littorina littorea*. In contrast, *Fucus edentatus* was markedly more preferred by urchins than *Ascophyllum*. This difference may partly explain their successional status since in the absence of ice perturbation, *Ascophyllum* is the dominant intertidal plant and replaces earlier colonization by *Fucus* spp. (Archambault and Bourget 1983).

One factor common to grazing resistant algae, which has not previously been emphasized, is the low degree to which they attract urchins. Urchins appear to detect and locate their preferred foods through chemosensory mechanisms. For example, Mann et al. (1984) demonstrate that urchins are attracted to *Laminaria longicruris* by low molecular weight substances released by the alga. Resistant algae may release smaller quantities of dissolved substances to reduce detection by urchins. Not producing or retaining such substances may involve morphological adaptations, such as less surface area or a thicker and less permeable cuticle.

Escapes in Space and Time

The grazing susceptible algae seem to survive by avoiding herbivores in either space or time. The multivariate analysis separated these algae into two distinct groups. The first consisted of the perennials, *Spongomorpha, Devaleraea, Laminaria,* and *Palmaria* (characterized by high consumption, high ash content, and intermediate attraction). *Spongomorpha* and *Devaleraea* occupy distinct zones in the low intertidal region and thus are usually isolated from urchins, however, *Laminaria* and *Palmaria* frequently occur near urchins in the subtidal zone. In eastern Canada, *Laminaria* spp. form localized beds where such factors as substratum barriers, water turbulence, or low salinities limit urchin grazing (Himmelman and Lavergne 1985), and form extensive subtidal beds where there have been mass urchin mortalities due to disease (Scheibling and Stephenson 1984; Miller 1985). In the northern Gulf of St. Lawrence where urchins are persistently abundant (on rocky substrata at Cap du Corbeau densities are ≈300 m^{-2}), *Laminaria* is generally confined to the algal fringe. *Palmaria* is sometimes epiphytic on the stipes of *Alaria,* and otherwise occasionally grows on rocks near low water level. All of the above four algae use spatial escapes to avoid grazing; they survive either where urchins are absent or where the effectiveness of urchin grazing is limited.

Of the second group of susceptible algae, *Chordaria, Scytosiphon, Ulvaria, Rhodomela,* and *Alaria* (characterized by high attraction, high dry mass, and intermediate consumption), the first three are opportunistic annuals. Much of the low intertidal region in the northern Gulf of St. Lawrence is severely ice-scoured during the winter. During the spring and summer, a variety of annual algae, including *Chordaria, Scytosiphon, Dictyosiphon foeniculatus,* and *Eudesme virescens*, rapidly colonize denuded surfaces (otherwise occupied by *Fucus, Spon-*

TABLE 5. Percentage dry mass, ash content, calorific content, and phenolic content of the various grazing susceptible and grazing resistant algae. The standard error is indicated in parenthesis ($n = 0$ in all cases except for the ash content of *Laminaria digitata* and *Scytosiphon lomentaria* where $n = 9$ and 4, respectively).

Species	Dry mass (percent wet mass)	Ash content (percent dry mass)	Energy content (Kcal·g^{-1} ash free dry mass)	Phenolic content (mg·g^{-1} wet mass)
		Grazing susceptible algae		
Chlorophyceae				
Ulvaria obscura	26.3 (0.29)	16.8 (0.30)	3.59 (0.049)	0.27 (0.037)
Spongomorpha arcta	14.0 (0.42)	32.6 (0.83)	4.55 (0.032)	0.02 (0.002)
Phaeophyceae				
Alaria esculenta	17.6 (0.31)	23.8 (0.74)	3.99 (0.035)	0.38 (0.077)
Ascophyllum nodosum	15.1 (1.35)	27.6 (1.42)	4.19 (0.047)	0.40 (0.038)
Chordaria flagelliformis	17.9 (0.11)	25.2 (0.18)	4.27 (0.045)	0.31 (0.048)
Fucus edentatus	13.8 (0.92)	27.9 (0.86)	3.28 (0.046)	0.62 (0.055)
Laminaria digitata	12.6 (0.32)	35.0 (0.53)	4.59 (0.032)	0.13 (0.015)
Saccorhiza dermatodea	8.16 (0.20)	48.3 (1.13)	3.20 (0.049)	0.14 (0.050)
Scytosiphon lomentaria	20.5 (0.26)	26.6 (3.56)	4.06 (0.034)	0.16 (0.009)
Rhodophyceae				
Devaleraea ramentacea	14.4 (0.36)	29.0 (0.51)	4.14 (0.049)	0.02 (0.001)
Palmaria palmata	15.8 (0.51)	27.4 (0.93)	4.14 (0.030)	0.03 (0.003)
Porphyra miniata	19.9 (0.61)	20.5 (0.89)	4.15 (0.034)	0.05 (0.003)
Rhodomela confervoides	21.8 (0.19)	23.0 (0.27)	3.72 (0.036)	0.18 (0.006)
		Grazing resistant algae		
Phaeophyceae				
Agarum cribrosum	19.3 (0.45)	24.1 (0.95)	3.70 (0.033)	0.85 (0.167)
Desmarestia viridis	14.3 (0.48)	20.9 (0.66)	2.75 (0.030)	0.11 (0.017)
Rhodophycaea				
Phycodrys rubens	22.0 (0.42)	25.5 (0.81)	3.82 (0.032)	0.03 (0.001)
Ptilota serrata	25.2 (0.35)	22.9 (0.30)	4.92 (0.033)	0.03 (0.001)
Turnerella pennyi	22.8 (0.34)	23.6 (0.45)	4.26 (0.042)	0.03 (0.001)

gomorpha, and *Devaleraea*). Whereas *Chordaria* and *Scytosiphon*, are primarily intertidal algae, *Ulvaria* also commonly colonizes the subtidal zone. In fact, when Himmelman et al. (1983a) removed urchins from a subtidal site in the St. Lawrence Estuary, *Ulvaria* formed a dense cover within 1 mo. Sporadically, *Ulvaria* naturally colonizes the zone below the algal fringe (Table 1), evidently due to decreases in urchin grazing. By contrast, *Rhodomela* and *Alaria* are perennials which grow mainly in the subtidal zone. *Rhodomela*, the most rapidly consumed of algae tested, is most commonly found near the upper limit of *Alaria* where urchins are absent. The *Alaria* zone is adjacent to the highest concentrations of urchins and a strong interaction exists between the two. In the winter, coincident with reduced urchin feeding rates, colonization by *Alaria* extends the fringe to greater depths, whereas in the spring and summer, when urchin feeding rates are high and grazing is more effective due to calmer conditions, grazing aggregations of urchins reduce the fringe to shallower depths (Himmelman 1985). *Alaria* is only consumed at an intermediate rate but it strongly attracts urchins; the latter probably explains the aggregating of urchins just below the *Alaria* fringe. The stipes are the last portions of older *Alaria* plants to be attacked (Himmelman 1985), probably because of their increased toughness and possibly due to the high phenolic content of the cortex (Pedersen 1980). Whereas the algae in this group of grazing susceptible algae share many characteristics, they show varied strategies against herbivores, including escapes in space and time.

Grazing resistant algae survive amongst urchins; nevertheless, their distribution and abundance is reduced by urchin grazing. For example, when urchins are experimentally removed, resistant algae, as well as susceptible algae, markedly increase in abundance (Himmelman et al. 1983a). Thus, chemical and morphological adaptations against grazing are only partial defenses. The association of resistant algae with boulders demonstrates an escape in space (Table 2). On boulders, as well as on vertical bedrock faces, urchin attacks are less effective because movement of the fronds by wave action (for such algae as *Agarum* and *Desmarestia*) is accentuated. The smaller grazing resistant reds often occur under *Agarum* and probably benefit from the protection offered by the movement of *Agarum* fronds.

Adaptive Strategies of Algae

In spite of the diversity of the defenses against herbivores among the common algae of the northern Gulf of St. Lawrence, the species tend to separate along a spectrum defined by two opposing survival strategies. In habitats where the intensity of grazing is null or low one finds the opportunists, such as *Chordaria*, *Ulvaria*, and *Saccorhiza* (and probably *Dictyosiphon foeniculatus*, *Chorda tomentosa*, and *Petalonia fascia*), which are characterized by strong colonization abilities and rapid growth. Being highly susceptible to grazing, they survive by escaping grazing in space or time. The opposing strategy is that of such algae as *Ptilota*, *Turnerella*, and *Phycodrys* (probably

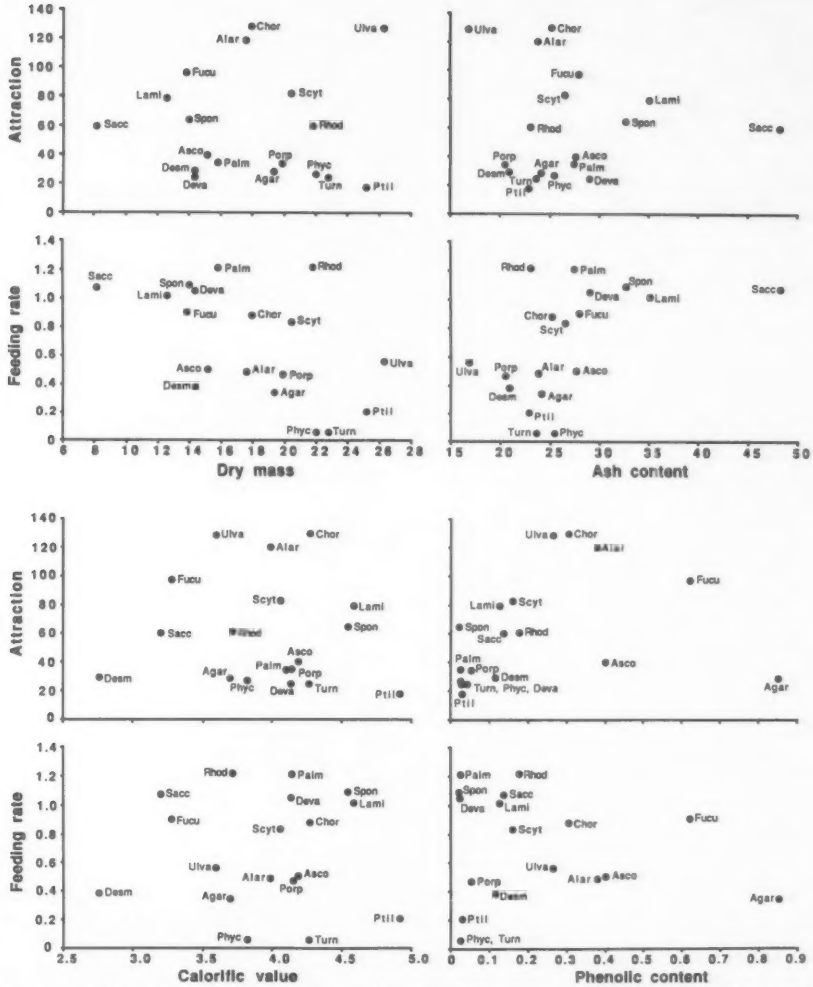

FIG. 7. Relation of attraction (urchin·0.25 m^{-2}) and feeding rate (g wet mass·urchin^{-1}·d^{-1}) to algal properties: percentage dry mass; percentage ash content·g dry mass^{-1}; calorific content (Kcal·g ash free dry mass^{-1}); and percentage phenolic content (mg·g wet mass^{-1}). (Complete algal names in Table 3).

also *Callophyllis cristata* and *Membranoptera alata*) which are found in intensely grazed habitats. They have low productivity and slow growth rates (Mathieson and Norall 1975; Himmelman et al. 1983a), possibly because they invest energy into substances or morphologies that reduce grazing. The most extreme example of this strategy is that of the crustose coralline algae (calcification). Other algae, such as *Alaria, Laminaria, Devaleraea, Palmaria,* and *Fucus,* have evolved strategies permitting some tolerance of grazing while having intermediate to high growth rates. The brown algae of this group are strongly competitive and relatively long-lived species (Buggeln 1974; Breen and Mann 1976; Himmelman et al. 1983a; Keats et al. 1984b; Chapman 1986; Johnson and Mann 1988). *Agarum* approaches the grazing resistant reds in this model, because of its slow growth rate compared with other brown algae (Vadas 1968; Mann 1972) and its relatively high resistance to grazing. *Desmarestia viridis* is an exception in this model because it is opportunistic and yet resists grazing. It colonizes in the winter and shows a rapid growth during the spring and summer, only to degenerate during the autumn. Its rapid growth suggests that it does not make marked energetic investments for increased grazing resistance. Probably its defenses, potentially the production of sulphuric acid (chemical defense) and the finely branched structure (morphological defense), do not require substantial costs.

Our model shares similarities with that proposed by Grime (1977), applied to terrestrial plants and subsequently applied to marine systems (Littler and Littler 1984). Taking into account the two types of external factors limiting plant abundance, stress (lack of light, water, or nutrients) and disturbance (physical or biotic), he proposes that three survival strategies have evolved: (1) the opportunistic strategy adopted by species in environments of high disturbance and low stress, (2) the stress-tolerant strategy, adopted in environments of high physiological stress

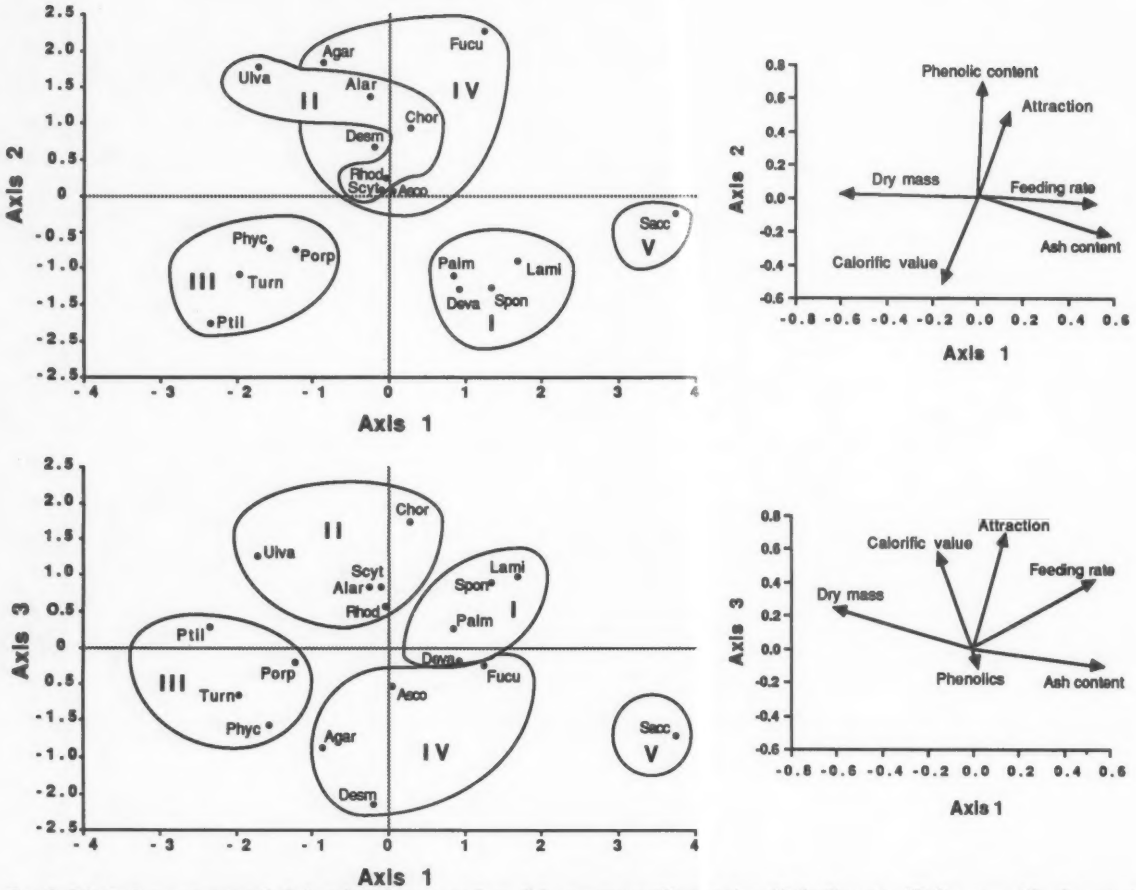

FIG. 8. Principal component analysis based on the mean values of the parameters (1) attraction, (2) feeding rate, (3) dry mass, (4) ash content per gram dry mass, (5) calorific value per gram ash free dry mass, and (6) phenolic content per gram ash free dry mass. Relative positions of the 18 algae along Axes 1 and 2 (above) and Axes 1 and 3 (below). The graphs to the right show the position of the six descriptors on the same axes. The algae are grouped according to their proximity in four dimensional space.

and low disturbance, and (3) the competitive strategy, developed in environments of low stress and low disturbance. Grime (1977) considers that conditions of high disturbance and high stress are not viable and that no plants have adapted to these conditions. Both our model, and that of Grime (1977), recognize groups of opportunistic and competitively superior species. Many intertidal algae probably fall into Grime's group of stress-tolerant plants, because they are subjected to decreased biotic disturbance but high physiological stress (Littler and Littler 1984). Our group of grazing resistant red algae, however, does not correspond to any of Grime's groups. Whereas Grime predicts that plants subjected to intensive disturbance by grazing should be opportunists, the resistant reds were slow growing perennials. We consider that resistant red algae are adapted to high stress as well as high disturbance, and thus belong to a fourth group which Grime considered non-viable. As well as tolerating intensive grazing, the red algae from the urchin dominated zone are well adapted to low light conditions. For example, *Ptilota, Turnerella, Callophyllis,* and *Phycodrys* penetrate to greater depths than most algae (well exceeding the penetration of all green and brown algae as well as many reds (J. H. Himmelman and H. Nédélec pers. observ; Mathieson 1979; and South and Hooper 1980). Further evidence of their adaptation to low light conditions is their colonization under dense canopies of large brown algae (Himmelman et al. 1983a). Superior ability to tolerate low light conditions is generally characteristic of rhodophytes and this probably contributes to their success in deeper water. Increased costs due to adaptations to high disturbance (intensive grazing) and high physiological stress (low light conditions) may account for the reduced growth, fecundity, and competitive ability of these algae.

The urchin, *Strongylocentrotus droebachiensis,* has a pronounced seasonal feeding cycle which is inverse to the reproductive cycle. Thus, its feeding rate decreases progressively as gonadal growth proceeds during the autumn and winter (even when surplus preferred algae are available), and increases threefold after spawning in the spring (Himmelman 1969, 1985; Larson et al. 1980). Thus, algae could synchronize rapid growth with periods of low urchin feeding as a strategy to limit urchin predation. This hypothesis is supported by the unusual characteristic of many subtidal algae of the northwestern Atlantic to accelerate their growth during the winter. For example, this

has been reported for most grazing resistant algae (*Phycodrys rubens, Callophyllis cristata, Neodelsia integra, Turnerella pennyi, Agarum cribrosum,* and *Desmarestia viridis*), for the major subtidal and low intertidal perennial species (*Alaria esculenta, Laminaria longicruris, L. digitata, L. saccharina,* and *Devaleraea ramentacea*) as well as for several opportunistic species (*Chorda tormentosa, Petalonia fascia, Scytosiphon lomentaria, Chordaria flagelliformis,* and *Ulvaria obscura*) (Mann 1972; South and Hooper 1980). Thus, the ability to grow rapidly under minimum annual temperatures and short photoperiod may have evolved as a temporal escape against urchin grazing.

A number of North Atlantic algae have encrusting or boring life stages in addition to the more familiar erect foliose or filamentous life forms and the two morphs may be ecologically distinct. Lubchenco and Cubet (1980) propose that such heteromorphic life cycles have been selected for by environments of variable grazing intensity; the upright stages are adapted for high growth and reproduction when grazing pressure is low, whereas the encrusting stages have lower rates of growth and reproduction and are adapted for surviving through periods of intensive grazing. Thin algal crusts, formerly referred to as *Ralfsia*, have been shown to be alternate stages of *Scytosiphon lomentaria* and *Petalonia fascia* (Wynne and Loiseaux 1976). Lubchenco and Cubet's (1980) report that in the intertidal zone in New England, the ralfsioid form predominates during the summer when there is intensive *Littorina* grazing, whereas *Scytosiphon* and *Petalonia* predominate during the winter when there is reduced grazing pressure (as well as in the summer when grazers are excluded). However, from studies on the Pacific coast of North America, Dethier (1981) states that the ralfsioid stage (while being resistant to grazing) is opportunistic, short lived, and most abundant in the winter with *Scytosiphon* and *Petalonia*. Dethier (1981) considers that the ralfsioid stage differs from *Scytosiphon* and *Petalonia* mainly in that it is more tolerant of physiological stress and physical disturbance. In the Mingan Islands, *Scytosiphon* forms luxuriant growths during the summer (temperatures are low and grazing by *Littorina littorina* is not severe due to its low abundance) but is strongly reduced by ice scouring during the winter. We hypothesize that here the ralfsioid stage is mainly important in maintaining intertidal *Scytosiphon* populations following ice scouring.

Porphyra spp. are also heteromorphic algae, there being a *Conchocelis* stage which bores into calcareous substrata and which is probably resistant to grazing. *Porphyra miniata* occurs sporadically in the urchin dominated subtidal zone. It develops from conchospores released by the *Conchocelis* stage (Chen et al. 1970). In our sites, *Turnerella pennyi* was the most common erect subtidal alga having an encrusting stage (*Cruoria*-like crust; South et al. 1972). On the basis of Lubchenco and Cubit's (1980) model, one would predict that the foliose morph would predominate in the winter when urchin feeding is reduced. However, the available observations do not suggest a marked seasonal pattern in the occurrence of either morph (South et al. 1972). The encrusting or boring stages of heteromorphic algae are generally competitively inferior (Lubchenco and Cubit 1980; Dethier 1981), and like the crustose coralline algae (Steneck 1982), are grazing dependent as well as grazing resistant. Thus, in the absence of grazing they are overgrown by more competitive algae, or by the upright morphs of the same species. In the northwestern Atlantic region, another potentially grazing resistant and grazing dependent alga is the non-calcareous encrusting red alga, *Peyssonnelia rosenvignii*, for which there is no known foliose stage. It replaces the calcareous encrusting reds at greater depths (J. H. Himmelman, pers. observ.).

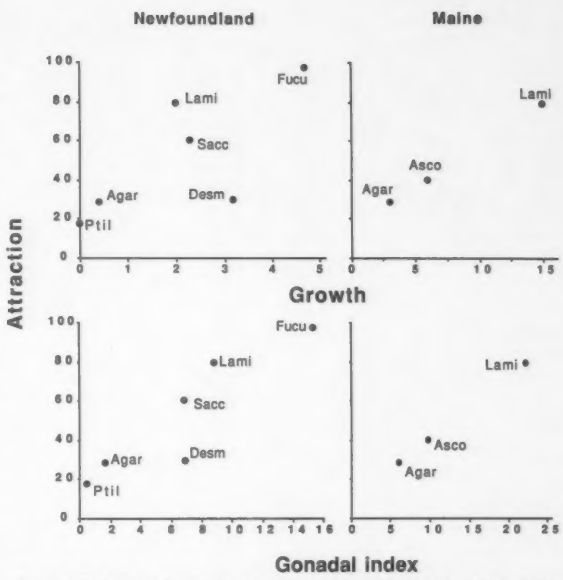

FIG. 9. Relation of attraction (urchin·0.25 m^{-2}) with the algal ability to support somatic growth (weight gain in grams) (above), and to support increases in the gonadal index (percentage gonadal weight) (below). (Complete algal names in Table 3). The data on somatic and gonadal growth from Newfoundland are from Keats et al. (1980; the urchins were maintained on single algal diets from early July to late August), whereas those from Maine are from Larson et al. (1980; somatic growth was measured from March to December 1975; gonadal growth from March 1975 to March 1976).

Urchin Food Preferences and Fitness

As food preferences probably represent adaptations for increasing the intake of foods that enhance fitness (Vadas 1977), correlations should exist between algal parameters and food preferences. Whereas calorific value provides a general measure of the potential value of a food source, it was not related to urchin preferences for these 18 common eastern Canadian macrophytes. This corroborates previous studies comparing herbivore food preferences and the calorific value of their foods (Paine and Vadas 1969; Nicotri 1980; Littler and Littler 1984; Steinberg 1985; Coen 1988), including studies on green sea urchins in the northeastern Pacific (Vadas 1977), and in the northwestern Atlantic (Larson et al. 1980). It is more likely that food value is related to the types of nutritive substances present (Himmelman and Carefoot 1975; Lowe and Lawrence 1976; Larson et al. 1980) and to the costs of detoxifying foods (Vadas 1977).

Data from several studies permit us to examine the effects of food preferences on rates of somatic and gonadal growth of the urchins. Vadas (1977) and Larson et al. (1980) emphasize the use of feeding rate as a measure of the food preference, and demonstrate that feeding rate correlates positively with somatic and gonadal growth. The data given by Keats et al. (1983) show a significant positive correlation between the rates of feeding

and either gonadal or somatic growth when urchins are provided with seven different algae (*Lithothamnium, Ptilota, Agarum, Desmarestia, Saccorhiza, Laminaria,* and *Fucus*) ($r_s = 0.61$, $P < 0.05$ for gonadal growth; $r_s = 0.74$, $P < 0.05$ for somatic growth). Our feeding rates tended to increase with the values for gonadal and somatic growth of Keats et al. (1983, 1984b) for the above algae except *Lithothamnium,* although not significantly so ($r_s = 0.60$, $P > 0.05$, for gonadal growth; $r_s = 0.54$, $P > 0.05$, for somatic growth).

Our field experiments demonstrated striking differences in the attraction of *S. droebachiensis* towards various algae (Fig. 6; Table 4). Our measures of attraction are positively related to the values given by Larson et al. (1980) for somatic and gonadal growth of urchins on three algae common to our studies (*Agarum, Ascophyllum,* and *Laminaria*; Fig. 9). Further, our measures of attraction were positively correlated with the data on gonadal indices for six different algae given in Keats et al. (1983, 1984b; $r_s = 0.94$, $P < 0.01$; Fig. 9). Attraction, as measured in our study, tended to be positively correlated with somatic growth as measured by Keats et al. (1983, 1984b) although not significantly ($r_s = 0.77$, $P > 0.05$). This correlation was stronger than with our data on feeding rate ($r_s = 0.54$, $P > 0.05$). We conclude that *S. droebachiensis* has evolved abilities enabling it to detect and locate algal foods which most enhance its fitness. These were not necessarily the most rapidly eaten or highest in energy but were generally characterized by high dry mass and intermediate phenolic content.

Emlen (1966) predicts that predators will forage indiscriminately when food is scarce and discriminately, with selection of the most predictable prey items, when food is abundant. In the case of *Strongylocentrotus droebachiensis*, strong food preferences are evident, even though food, other than coralline algae, is often rare. The subtidal region of the northern Gulf of St. Lawrence and, indeed, over vast areas of the northwestern Atlantic, provide striking examples of overgrazed environments, yet urchins have retained the ability to be selective feeders. When food is scarce urchins are not selective feeders, rather they feed on non-preferred foods such as *Ptilota* and calcareous algae. The urchin is extremely well adapted to food-limited environments, and displays numerous survival strategies, including reduction of metabolic activities (Johansen and Vadas 1967), resorption of gonads and even of somatic tissues (Ebert 1967; Vadas 1977), feeding on planktonic organisms (Leighton and Jones 1968) and dissolved organic matter (Pearse and Pearse 1971), and the possession of a gut flora capable of nitrogen fixation and of synthesizing essential amino acids (Guerinot et al. 1977; Fong and Mann 1980).

The above strategies allow survival but permit little or no production of gametes. Only individuals which obtain preferred food can grow and produce gametes and thus contribute to species survival (Vadas 1977; Keats et al. 1983, 1984b). This suggests that food preference is genetically determined. In the widespread urchin dominated areas of eastern Canada, preferred macroalgal foods can be obtained by two means, (1) by moving to the algal fringe, and (2) by waiting for drift algae from the shallow algal zone to fall to the bottom. Urchins have a remarkable ability to detect and locate favourable prey. For example, in our field experiments, within 24 h of placing preferred algae, *Chordaria, Ulvaria,* and *Alaria*, on the bottom, there was a five-fold increase in urchin density (from 23–29 urchins·0.25 m^{-2} in the control samples to 120–130 urchins·0.25 m^{-2} in the vicinity of preferred algae). In the field, medium-sized to large urchins readily aggregate on drift algae, thus demonstrating the importance of this opportunistic mode of feeding. Urchins from the barren zone often have fragments of preferred algae in their guts, algae which do not grow in this zone (J. H. Himmelman and H. Nédélec pers. observ.). If urchins on barrens contribute to reproductive survival, it must be due to the use of drift algae. The appearance of drift algae in the barrens is sporadic (probably greatest in autumn and winter coincident with heavy storms and ice abrasion) and the urchin's acute abilities to detect and locate such foods are undoubtedly advantageous in exploiting this resource. Concentrations of medium to large-sized urchins are also frequent in the zone adjacent to the algal fringe (Fig. 3), probably because they are attracted by *Alaria* and other fringe algae. Thus, detection and movement towards occasional food sources appears a major element in the urchin's foraging strategy, and promotes intake of foods that enhance fitness.

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Heterotrophic Bacterial Production and Its Dependence on Autotrophic Production in a Hypertrophic African Reservoir[1]

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The incorporation of [methyl-^{3}H]thymidine (TdR) into bacterial DNA in Hartbeespoort Dam, South Africa was measured over 16 mo and at nine depths. Bacterial numbers at the surface ranged between 2.45 and 32.20 $\times$ 10^6 cells·mL^{-1} ($\bar{x}$ = 8.83 $\times$ 10^6 mL^{-1}) while bacterial production varied between 1.0 and 251 pmol TdR·L^{-1}·h^{-1} (0.01 to 1.9 mg C·m^{-3}·h^{-1}). At the bottom, production ranged between 0 and 26.7 pmol TdR·L^{-1}·h^{-1} (0–0.2 mg C·m^{-3}·h^{-1}). The fastest bacterial doubling time was 59 h. At the surface, bacterial production was dominantly correlated to chlorophyll *a* (6.6–6530 mg·m^{-3}) and phaeopigments (0.9–378 mg·m^{-3}) (r = 0.81) followed by primary production (26.6–8886 mg C·m^{-3}·h^{-1}) (r = 0.77) (n = 30–34, p<0.001). However, below 5 m, water temperature and bacterial numbers were the dominant correlates. Bacterial production for the water column averaged 2% of daily, areal primary production. The data demonstrated a close coupling between autotrophic production and heterotrophic bacterial production. However, the low bacterial production compared with primary production, together with the small size of the bacteria (usually 0.09–0.25 μm width), suggest substrate supply was a major limiting factor of bacterial growth.

Nous avons mesuré l'incorporation de [méthyl-^{3}H]thymidine (TdR) dans l'ADN de bactéries du réservoir de Hartbeespoort, en Afrique du Sud, durant une période de 16 mo et à neuf profondeurs. La quantité de bactéries à la surface variait de 2,45 à 32,20 $\times$ 10^6 cellules·mL^{-1} ($\bar{x}$ = 8,83 $\times$ 10^6·mL^{-1}) et la production bactérienne variait de 1,0 à 215 pmol TdR·L^{-1}·h^{-1} (0,01 à 1,9 mg C·m^{-3}·h^{-1}). Au fond de l'eau, la production variait de 0 à 26,7 pmol TdR·L^{-1}·h^{-1} (0–0,2 mg C·m^{-3}·h^{-1}). Le temps de doublement du nombre de bactéries le plus court était de 59 h. À la surface, la production bactérienne était principalement corrélée à la chlorophylle *a* (6,6–6530 mg·m^{-3}) et aux pigments bruns (0,9–378 mg·m^{-3}) (r = 0,81), suivis de la production primaire (26,6–8886 mg C·m^{-3}·h^{-1}) (r = 0,77) (n = 30–34, p<0,001). Aux profondeurs supérieures à 5 m, cependant, la température de l'eau et le nombre de bactéries étaient les principaux corrélats. La production bactérienne moyenne pour l'ensemble de la colonne d'eau était de 2 % de la production primaire quotidienne du secteur étudié. Nos données ont démontré qu'il y avait une relation étroite entre la production autotrophe et la production bactérienne hétérotrophe. Cependant, le fait que la production bactérienne était faible, comparée à la production primaire, et la petite taille des bactéries (0,09–0,25 μm de largeur, habituellement) laissent penser que les ressources nutritives limitaient pour une bonne part la croissance bactérienne.

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Bacteria are generally accepted as the prime decomposers of organic carbon and regenerators of minerals in aquatic ecosystems. A number of studies have shown a close coupling between heterotrophic bacterial activity and growth and phytoplankton primary production, especially associated with the production of dissolved organic carbon. As noted by Wright (1988), it has been assumed for some time that aquatic bacterial populations were controlled by the availability of substrate, often existing in a semi-starved or nongrowing state, and were changing only slowly. More recently, the potential importance of bacteria as a link between dissolved organic carbon and the classical grazing food chain has been recognized. The quantitative importance of this link has been questioned (Ducklow et al. 1986) and the bacterial role remains unresolved (Wright 1988).

Bird and Kalff (1984) and Robarts (1988) have shown that bacterial numbers in aquatic systems do not increase as rapidly as algal biomass with increasing nutrient concentrations. Bird and Kalff suggested this need not signify a smaller role for bacteria in enriched systems if bacterial production per unit bacterial biomass increased as total bacterial biomass increased. Estimates of bacterial production are therefore essential for assessing the importance of heterotrophic bacteria in consuming primary production and in providing material and energy for higher trophic levels in pelagic ecosystems (Kirchman and Hoch 1988).

Cole et al. (1988) have noted that the factors which regulate aquatic bacterial production are a matter of considerable speculation. According to Cole et al. the literature suggests bacterial production is controlled by, or is directly related to, the supply of decomposable organic matter but little quantitative data exist to support this notion. If this is correct, bacterial production in

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different systems should be correlated with the supply or standing stock of decomposable organic matter which, while presently impossible to quantify precisely, can be indirectly estimated by measures of variables such as primary production (Cole et al. 1988). From their review of freshwater and marine data, Cole et al. found that on an areal basis for the entire water column, bacterial production averaged 30% of water column primary production.

Hartbeespoort Dam is a hypertrophic, warm, monomictic reservoir (Robarts et al. 1982) with the *Microcystis aeruginosa*-dominated phytoplankton having the highest recorded rates of primary production (Robarts 1984). While there are an increasing number of published studies of bacterial production in marine and freshwaters, none has been published for a system in which the phytoplankton standing crops and production compares with Hartbeespoort Dam. The objectives of our study were to determine the factors controlling bacterial production and the extent of bacterial utilization of the extremely high phytoplankton carbon production in the water column. Eutrophication remains a major environmental issue in many countries. While Hartbeespoort Dam is an extreme example of a hypertrophic system, our data provide new insights on the impact of high nutrient loads on bacterial populations and food webs, a subject which is largely unknown.

Materials and Methods

The study was carried out at a permanently buoyed station in the main basin of Hartbeesport Dam. Water samples were collected at biweekly intervals between January 1987 and April 1988 with a 6-L Van Dorn water sampler.

Water temperature was measured with a Cole-Parmer 8502-20 thermistor at 0, 0.5, and then 1-m intervals to the bottom. Dissolved oxygen was measured at the same depths to the aerobic–anaerobic interface by the azide modification of the Winkler technique and determined spectrophotometrically (Ashton and Twinch 1985). These data are given as percent saturation and were corrected for water temperature and altitude (~1200 m a.m.s.l.). The dissolved organic carbon concentration (DOC) was measured at 5-m intervals using a Beckman 915A TOC analyzer following $K_2S_2O_8$ and UV irradiation digestion (Van Steenderen and Lin 1981).

The attenuation of photosynthetically available radiation (PAR, 400–700 nm) was measured with a quantum sensor (Lambda Instruments, LI-185 and LI-125 sensor). Readings were taken from just below the surface and at 25–50-cm intervals to the depth of 1% of the surface value, the depth of the euphotic zone.

Immediately upon collection of the water samples, 50–2000-mL subsamples were drawn through GF/C glass fibre filters. The filters were folded and placed in opaque vials containing 90% ethanol. After returning to the laboratory, the vials were placed in 78°C water for 5 min. The extracted chlorophyll *a* and phaeopigments were determined spectrophotometrically (Zohary and Robarts 1989).

Primary production (phytoplankton particulate organic carbon production) and the production of ^{14}C-labelled extracellular dissolved organic carbon (EDOC) were measured as described by Robarts and Sephton (1989). Measurements spanned the euphotic zone. Two light bottles and one dark bottle were injected with 74 kBq of $NaH^{14}CO_3$ (The Radiochemical Centre, Amersham, U.K.) and resuspended in the water at the depths from which the samples were obtained for 1.5 to 2.5 h. At the end of the incubation, 5–50-mL subsamples, depending on the chlorophyll concentration, were filtered through 0.45-μm pore size membrane filters which were placed in glass scintillation vials containing 0.5 mL of 0.5 M HCl. After 2 h, 10 mL of Filter Count (Packard Instruments Ltd.) were added and the vials counted once the filters completely dissolved. In samples in which the chlorophyll concentration exceeded 100 $mg \cdot m^{-3}$, the algal material was first treated with tissue solubilizer before the fluor was added. From the filtrates of the light bottle samples, 5-mL subsamples were taken for EDOC determinations, placed in glass vials containing 0.5 mL of 0.5 M HCl and allowed to stand open overnight. This removed unincorporated ^{14}C. The next morning 5 mL of hydrophilic scintillator were added. Background, or blank, samples were prepared using the filtrates from the 4- and 5-m dark bottles. The mean value of these filtrates were subtracted from the light bottle values at all depths.

Water samples for the determination of bacterial biomass, numbers, and production were collected from 0, 0.5, 1, 3, 5, 10, 15, and 20-m depths plus 1 m above the bottom (24–28 m). Water (10 mL) for bacterial enumeration was placed in sterile glass tubes and preserved with Lugol's iodine solution (Pomroy 1984). Total bacterial counts for each sample were made using an epifluorescence microscope and DAPI (4′,6-diamidino-2-phenylindole) stain (Robarts and Sephton 1981).

During the course of the present study, and earlier work spanning 6 yr, samples were selected from the aerobic and anaerobic zones for bacterial volume estimates. Bacteria stained with DAPI were photographed and the resulting slides were projected on to a screen. Cell dimensions were measured with an electronic digitizer. Additional water samples were collected and preserved with glutaraldehyde. Bacteria in these samples were prepared for scanning electron microscopical examination with the method of Paerl and Shimp (1973).

For an independent assessment of bacterial sizes in Hartbeespoort Dam, a series of photographic negatives of DAPI stained bacteria (mixture of samples preserved with Lugol's or formalin) were examined by Dr. C. Krambeck (Max-Planck-Institut für Limnologie, West Germany). The size distribution and cell volume of free-living bacteria was obtained by projection of these negatives onto a digitizer field, followed by image analysis using the digitizer-microcomputer system described by Krambeck et al. (1981).

Bacterial production was determined from the rate of [*methyl*-^3H]thymidine incorporation into bacterial DNA. Water samples of 10 mL were transferred to sterile screw-top polystyrene tubes. In the case of anaerobic samples, water was allowed to fill the tubes and then overflow in order to exclude oxygen. For each depth there were two live and one control tube. Controls were prepared by the addition of 0.5 mL of 5M NaOH (Wicks and Robarts 1987).

Working solutions (~3700 $kBq \cdot mL^{-1}$) of [*methyl*-^3H] thymidine (2812–3367 $GBq \cdot mmol^{-1}$, New England Nuclear, Boston, USA; The Radiochemical Centre, Amersham, U.K.) were made daily with distilled water which had been sterilized using 0.2-μm pore size membrane filters. Labeled thymidine was added to each incubation tube to give a final thymidine concentration of 20 nM in order to saturate bacterial uptake (Robarts et al. 1986). The tubes were then attached to an incubation harness and returned to the depths from which they had originated. Incubation time was 30 min during summer (September–March) and 60 min in winter (April–August).

Thymidine incorporation was stopped, and the samples preserved, by the addition of 0.5 mL of 5M NaOH. The tubes were then stored on crushed ice until they could be processed in the laboratory usually within 3 h, but always within 24 h, of NaOH addition to the samples. Labelled DNA recovery is not affected by 0.25 M NaOH at 4°C for 24 h (Wicks and Robarts 1987).

In the laboratory, labelled DNA was extracted and purified using the method of Wicks and Robarts (1987). The labelled DNA was precipitated in ice-cold TCA, collected on 0.2-μm pore size cellulose nitrate membrane filters, washed with phenol-chloroform (50% w/v) and then 80% ethanol. After removing the non-filtering area, the filters were placed in 10 mL of Filter-Count (Packard Instruments, The Netherlands) which completely dissolved the filters. Tritium incorporation into DNA was determined with a liquid scintillation counter and counts were corrected for quench with an external standard and for machine counting efficiency.

Bacterial growth rates measured as moles of thymidine (TdR) incorporated into DNA were converted to bacterial carbon production with two equations due to the uncertainty associated with key variables:

$$\text{Production (mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}) = \text{TdR} \times 318 \times {}^{1}/_{p} \times \text{C/DNA},$$

where TdR = mol thymidine incorporated into DNA, 318 = average molecular weight of the four nucleotides in DNA, p = proportion of thymine in DNA (average = 0.25), C/DNA = 5 for small aquatic bacteria (Fuhrman et al. 1986).

$$\text{Production (mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}) = \text{TdR} \times F \times \text{Vol.} \times \text{C/Vol.}$$

where TdR = mol thymidine incorporated into DNA, F = factor for converting mol TdR incorporated into DNA to number of cells produced (1×10^{18} cells·mol^{-1}; Moriarty 1988). Vol = average volume of bacteria (0.013 μm^{-3}, see below,) C/Vol = carbon per unit volume of bacteria of 560 fg C·μm^{-3} (Bratbak 1985).

Integrated bacterial production for the euphotic zone and for the water column were obtained planimetrically from production versus depth plots.

Chin-Leo and Kirchman (1988) have recommended that coincident measurements of bacterial protein labelling by [^{3}H]leucine provides an independent check of the thymidine method. We followed the changes in bacterial numbers, DNA labelling by thymidine and protein labelling by leucine over a diel cycle in Hartbeespoort Dam. Water was collected from the upper 1 m of the lake and 10 L were poured into dialysis tubing. The tubing was knotted then attached to a float to incubate the enclosed water at about 0.5 m in the water column. Subsamples of the water were immediately collected for bacterial numbers and estimates of bacterial production using labelled thymidine as described above. Separate samples were also taken to measure the rate of labelled leucine incorporation into bacterial protein as described by Wicks and Robarts (1988). Subsamples of water for these three measurements were taken at various intervals over 24 h (see Fig. 4).

Working solutions (ca. 3700 kBq·mL^{-1}) of *L*-[4,5-^{3}H]leucine (2220 GBq·mmol^{-1}; DuPont, NEN Research Products, Boston, Mass.) were made daily following the procedure above for thymidine. Labeled leucine was added to two experimental plus one control 10-mL water samples to give a final leucine concentration of 20 nM. Samples were incubated for 30 min at the same depth as the dialysis bag. After incubation, the reactions were stopped by the addition of 100% TCA to a final concentration of 5%. Controls were prepared by the addition of labeled leucine followed immediately by the addition of TCA. Samples containing 5% TCA were stored on ice until the second day of the experiment. They were then placed in boiling water for 30 min and then cooled in ice for 15 min. The TCA-precipitated macromolecules were collected on membrane filters and washed with 5 mL of 5% TCA followed by 5 mL of cold 80% ethanol (Wicks and Robarts 1988). The nonfiltering area of the filters was removed and the filters were placed into scintillation fluor and assayed for ^{3}H content as described above for thymidine.

Statistical analyses of the data were done using SPSS (Statistical Package for the Social Sciences; Hull and Nie 1981).

Results

Surface water temperatures in Hartbeespoort Dam ranged between 12.1 and 28.2°C ($\bar{x}$ = 22.2°C) during the study period. The water temperature 1 m above the sediments (24–28 m depth, depending upon seasonal fluctuations between a wet summer and a dry winter) had a range of 11.3 to 21.8°C with a mean value of 17.1°C. The reservoir stratified in September with the start of summer and underwent holomixis in April at the beginning of winter.

Surface oxygen concentrations, as percentage saturation, ranged between 6.4 and 224% ($\bar{x}$ = 112%). The lower values are characteristic of this reservoir at overturn (Robarts 1985). Oxygen saturation exceeding 100% occurred down to 5 m where the range was 7.3–134% ($\bar{x}$ = 84%). With the onset of stratification, the hypolimnion became anaerobic, extending upward to within 9 m of the lake surface (Fig. 1). In mid-summer, H_2S was noticeable in the water samples below 20 m.

The DOC concentration in Hartbeespoort Dam was relatively uniform with depth during the study period. The highest mean concentration of 8.5 mg C·L^{-1} (range 6.6–16.3 mg C·L^{-1}) was measured at the surface while the lower value of 7.1 mg C·L^{-1} was recorded at 20 m (range 5.3–10.7 mg C·L^{-1}). The highest measured DOC concentration of 24.8 mg C·L^{-1} was recorded at the bottom while the lowest value of 4.3 mg C·L^{-1} was found at 15 m.

Microcystis aeruginosa was the dominant autotroph from November to July due to its ability to track diurnal mixed layers (Zohary and Robarts 1989). Fig. 1a shows that the phytoplankton biomass was strongly stratified with depth which was due to the buoyancy mechanism of *Microcystis*. The largest biomass occurred in April 1987 when a surface concentration of >6500 mg Chl*a*·m^{-3} was recorded. Below about 5 m the chlorophyll *a* (Chl*a*) concentration showed little change with depth or season.

The concentration of phaeopigments had both a depth and time pattern similar to chlorophyll *a*. The surface phaeopigment concentration ranged between 0.9 and 378 mg·m^{-3} ($\bar{x}$ = 24.7 mg·m^{-3}) while at depths of 3 m and greater the values ranged from <0.5 mg·m^{-3} to a maximum of 27 mg·m^{-3}. The mean concentrations ranged from 5.6 mg·m^{-3} at 3 m to 2.7 mg·m^{-3} at 15 m and below.

The euphotic zone depth in Hartbeespoort Dam ranged between virtually 0 m in April and 7.5 m in September 1987 (Fig. 2). Due to the steep biomass and light gradients with depth, primary production was limited to a narrow band usually less than about 2 m deep. Surface primary production ranged between 26.6 and 8886 mg C·m^{-3}·h^{-1} ($\bar{x}$ = 919 mg C·m^{-3}·h^{-1}) while at 5 m the range was 0 to 14.7 mg C·m^{-3}·h^{-1} ($\bar{x}$ = 3.4 mg C·m^{-3}·h^{-1}) (Fig. 2).

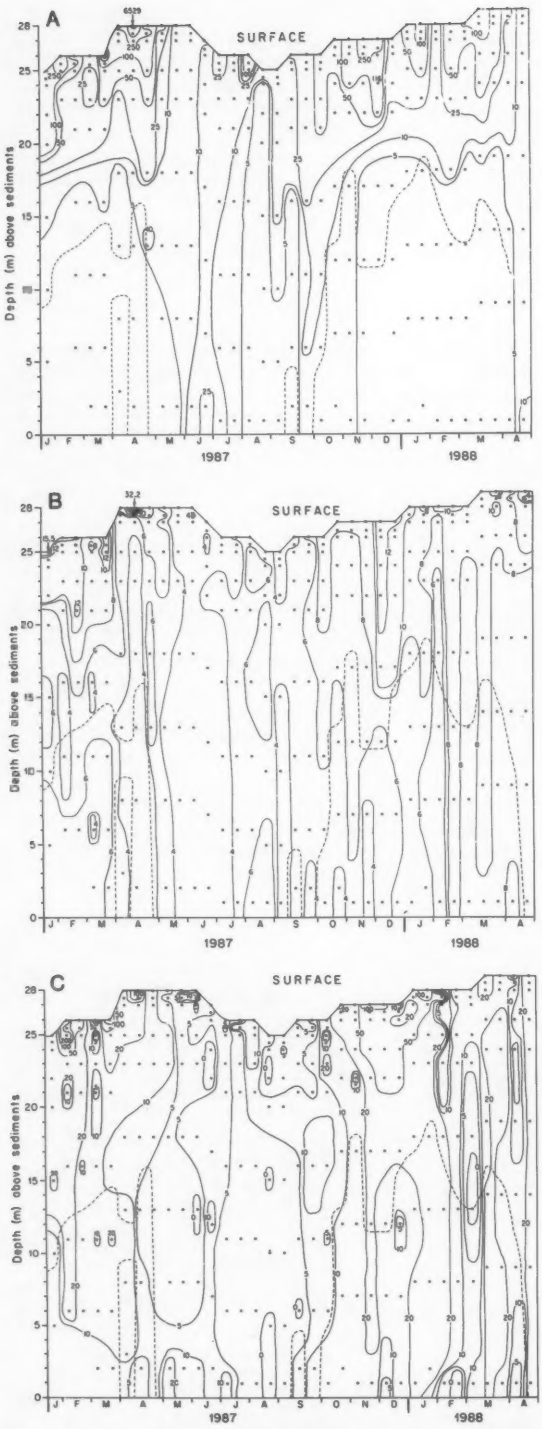

FIG. 1. Changes in (A) chlorophyll *a* ($mg \cdot m^{-3}$) concentration, (B) bacterial numbers ($\times 10^6$ cells$\cdot mL^{-1}$), and (C) the rate of thymidine incorporation into bacterial DNA (pmol TdR$\cdot L^{-1} \cdot h^{-1}$) with depth and time in Hartbeespoort Dam, South Africa. Broken line indicates the aerobic–anaerobic interface. Data have been plotted from the bottom to the surface to take into account the fluctuating water level.

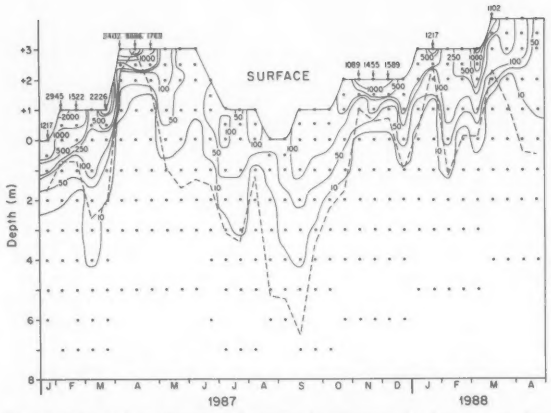

FIG. 2. Depth-time diagram showing the changes in the rate of phytoplankton production (mg $C \cdot m^{-3} \cdot h^{-1}$) and the depth of the euphotic zone (1% level of subsurface PAR, broken line) in Hartbeespoort Dam. Arrows at the surface indicate production rates in excess of 1000 mg $C \cdot m^{-3} \cdot h^{-1}$. Data have been plotted to show the changes in water level with the changing summer wet season and the winter dry season (reference point = reservoir capacity at lowest level).

The production of EDOC by the phytoplankton was usually low compared with the rate of carbon incorporation into biomass. EDOC production was usually greatest at the surface (range = 1.2–150 mg $C \cdot m^{-3} \cdot h^{-1}$, $\bar{x} = 25.1$ mg $C \cdot m^{-3} \cdot h^{-1}$) and decreased rapidly thereafter with a mean value of 2.0 mg $C \cdot m^{-3} \cdot h^{-1}$ (range 0–14.8 mg $C \cdot m^{-3} \cdot h^{-1}$) being recorded at 5 m.

The greatest numbers of bacteria (Fig. 1b) were usually associated with the upper water column similar to the chlorophyll and phaeopigment distributions. Bacterial numbers showed their greatest variation at the surface ranging between 2.45 and 32.20×10^6 cells$\cdot mL^{-1}$ ($\bar{x} = 8.83 \times 10^6 \cdot mL^{-1}$). At all other depths the range was about five-fold (<3.00 to $>14.00 \times 10^6$ cells$\cdot mL^{-1}$). Bacterial numbers were lowest in winter (July–August) followed by a steady increase to summer highs, similar to observations made in previous years (Robarts and Sephton 1984; Robarts 1988).

The bacterial cell size results from the SEM studies and those from the epifluorescent micrographs of DAPI-stained bacteria analyzed by ourselves and Dr. C. Krambeck (Max-Planck Institut für Limnologie, Plön, West Germany), all indicated that the Hartbeespoort Dam bacterial population was dominated by very small rods and cocci (maximum cell width <0.71 μm, with cell length averaging 2.15 × cell width). Some cells were less than 0.09 μm wide, whilst the majority of cells had widths between 0.09 and 0.25 μm. As a percentage of the total bacterial biomass, cells with widths of ≤ 0.18 μm contributed 19.3% while cells with widths >0.18 μm contributed 80.7%. Mean bacterial cell volume was 0.013 μm^3.

At the surface and 0.5-m depth, primary production and water temperature were the main factors correlated with bacterial numbers (Table 1). Between 1 and 10 m bacterial numbers were

TABLE 1. Summary of Spearman rank correlation coefficients between bacterial number (cells $\times 10^6 \cdot mL^{-1}$) and various environmental parameters in Hartbeespoort Dam, South Africa. For clarity only coefficients ≥ 0.4 are given. Number of cases is 30–34 for all depths except bottom (24–28 m) where it is 27–30. Two tailed probabilities are $p \leq 0.001$ for $r \geq 0.5$, $p \leq 0.02$ for $r \geq 0.4$. NS = not significant at $p > 0.05$, S = significant at $p < 0.05$ but $r < 0.4$, — = not measured, ND = not detected. EDOC = algal extracellular dissolved organic carbon production (mg $C \cdot m^{-3} \cdot h^{-1}$), DOC = dissolved organic carbon (mg $\cdot L^{-1}$).

Depth (m)	Water temperature	Percent O_2 saturation	Chlorophyll *a*	Phaeopigments	Primary production	EDOC	DOC
0	0.69	NS	0.56	0.56	0.70	0.40	NS
0.5	0.76	0.54	0.67	NS	0.80	0.47	—
1	0.77	0.40	0.45	NS	0.54	NS	—
3	0.76	0.44	0.56	S	NS	NS	—
5	0.62	NS	0.44	NS	NS	NS	NS
10	S	NS	NS	NS	ND	ND	NS
15	0.45	−0.52	NS	NS	ND	ND	NS
20	0.55	−0.60	−0.41	−0.45	ND	ND	−0.57
Bottom	0.43	−0.50	−0.45	NS	ND	ND	−0.47

dominantly correlated to water temperature and positive correlations between these two variables were found to the bottom. At 15 m and deeper, significant inverse correlations were found between bacterial numbers and oxygen saturation, chlorophyll *a* and DOC concentrations (Table 1). When the data for the different depths were pooled, bacterial numbers were most strongly correlated to water temperature ($r = 0.72$, $n = 299$, $p < 0.001$) followed by chlorophyll *a* concentration ($r = 0.45$, $n = 293$, $p < 0.001$).

Using multiple linear regression analysis, significant increases in the strongest bivariate correlation coefficients between bacterial numbers and environmental variables were found for the surface and for the entire data set only. For the surface, bacterial numbers correlated to primary production plus water temperature plus phaeopigments with an $r = 0.95$ ($n = 28$, $p < 0.001$). Bacterial numbers at all depths correlated with primary production plus water temperature plus phaeopigment concentration ($r = 0.83$, $n = 277$, $p < 0.001$).

The highest rates of bacterial production (pmol TdR $\cdot L^{-1} \cdot h^{-1}$ incorporated into DNA) in Hartbeespoort Dam were usually found at, or near, the surface and decreased with depth (Fig. 1c). Bacterial production ranged between 1.0 and 251 pmol TdR $\cdot L^{-1} \cdot h^{-1}$ ($\bar{x} = 42.4$ pmol TdR $\cdot L^{-1} \cdot h^{-1}$) at the surface while at the bottom, the mean rate was 10.8 pmol TdR $\cdot L^{-1} \cdot h^{-1}$ with a range of 0 to 26.7 pmol TdR $\cdot L^{-1} \cdot h^{-1}$ (Table 2). No measurable rate of DNA labelling occurred occasionally at all depths, except for the surface and 0.5 m. Bacterial production in the anaerobic zone was not markedly lower than in the aerobic zone below 1 m for any given date (Fig. 1c).

Bacterial carbon production, calculated using equation (1), ranged between 0 and 1.6 mg $C \cdot m^{-3} \cdot h^{-1}$. Table 2 gives the same data calculated with equation (2), which used a previously published conversion factor as we failed to obtain one using the method of Kirchman et al. (1982). These values ranged between 0 and 1.9 mg $C \cdot m^{-3} \cdot h^{-1}$. An analysis of variance (ANOVA) of these data indicated no significant difference between the rates of bacterial carbon production calculated with the two equations ($F = 1.49$, $p > 0.1$).

The rate of bacterial cell production in Hartbeespoort Dam ranged between 0 and 0.25×10^{12} cells $\cdot m^{-3} \cdot h^{-1}$ (Table 2). The maximum specific growth rate of $0.017 \cdot h^{-1}$ was measured at 1 m and corresponded to a population doubling time of 59 h. The maximum measured doubling time was 5000 h but on several occasions at depths >1 m no growth was measured.

The specific rate of thymidine incorporation into DNA per bacterial cell tended to be highest at 0.5 m where the mean value was 4.7×10^{-21} mol TdR $\cdot$ cell$^{-1} \cdot h^{-1}$ (range 0.2–20.8×10^{-21} mol TdR $\cdot$ cell$^{-1} \cdot h^{-1}$). The maximum specific rates of bacterial DNA labelling for all surface to 1-m samples approached 20×10^{-21} mol TdR $\cdot$ cell$^{-1} \cdot h^{-1}$, but at ≥ 3 m, the maximum rates ranged between 6.0 and 7.9×10^{-21} mol TdR $\cdot$ cell$^{-1} \cdot h^{-1}$, giving mean values of about 2.5×10^{-21} mol TdR $\cdot$ cell$^{-1} \cdot h^{-1}$.

At the surface, the chlorophyll *a* and phaeopigment concentrations, followed by primary production, were the dominant variables correlated with bacterial production (Table 3). Algal standing crop, water temperature, oxygen saturation and bacterial numbers were all correlated with bacterial production down to 5 m. Below 5 m, water temperature and bacterial numbers were the dominant correlates. At the bottom of the water column no significant correlation was found between bacterial production and any of the measured environmental parameters. The use of multiple linear regression added <0.1 to the *r*-values of the strongest bivariate correlations shown in Table 3. An exception was the data for 0.5 m where bacterial production was most strongly correlated with the chlorophyll *a* concentration plus water temperature ($r = 0.84$, $n = 32$, $p < 0.001$).

No significant correlation between bacterial production and primary production (mg $C \cdot m^{-3} \cdot h^{-1}$) was found below 1 m (Table 3). However, when the volumetric rates of primary production were replaced by the areal values for the euphotic zone (mg $C \cdot m^{-2} \cdot h^{-1}$) a positive correlation with bacterial production was calculated down to 20 m. The correlation coefficients ranged from 0.46 at 3 m to 0.36 at 20 m with $p < 0.02$. At the bottom of the reservoir, the correlation was not significant ($r = 0.24$, $n = 30$, $p > 0.1$).

The annual cycles of phytoplankton production in the euphotic zone of Hartbeespoort Dam are compared with seasonal bacterial production in the euphotic zone and entire water column (nmol TdR $\cdot m^{-2} \cdot h^{-1}$) in Fig. 3. Primary production ranged between 65 mg $C \cdot m^{-2} \cdot h^{-1}$ in August 1987 to 2900 mg $C \cdot m^{-2} \cdot h^{-1}$ in April 1987. Bacterial production in the euphotic zone varied between 9.8 nmol TdR $\cdot m^{-2} \cdot h^{-1}$ in June 1987 and 335.9 nmol TdR $\cdot m^{-2} \cdot h^{-1}$ in February 1987 corresponding to 0.1 and 2.1 mg $C \cdot m^{-2} \cdot h^{-1}$, respectively. As a percentage of primary production, euphotic zone bacterial production ranged between 0.01 and 1.1% ($\bar{x} = 0.1\%$), the latter value occurring at overturn in 1988 with the sharp drop in primary production

TABLE 2. Bacterial production in Hartbeespoort Dam as the rate of thymidine incorporation into DNA, as organic carbon and as cells produced calculated from equation (2). The specific growth rate and doubling times are also indicated. Data given are the mean and the range for each variable. ND = not detected.

Depth (m)	Bacterial production (nmol TdR·m^{-3}·h^{-1})	Cell production ($\times 10^{12}$·m^{-3}·h^{-1})	Specific growth rate (h^{-1})	Doubling time (h)	Carbon production (mg C·m^{-3}·h^{-1})
0	42.4	0.043	0.005	200	0.3
	1.0–251	0.001–0.251	0.0004–0.008	125–2500	0.01–1.9
0.5	40.1	0.040	0.005	167	0.3
	0.6–181	0.001–0.181	0.0002–0.014	71–5000	0.01–1.3
1	28.3	0.029	0.004	250	0.2
	0.0–211	0.0–0.211	0.0–0.017	59–ND	0.0–1.6
3	22.2	0.022	0.003	333	0.2
	0.0–70.9	0.0–0.071	0.0–0.005	200–ND	0.0–0.5
5	17.5	0.018	0.003	333	0.1
	0.0–60.3	0.0–0.061	0.0–0.004	250–ND	0.0–0.5
10	15.3	0.016	0.003	333	0.1
	0.0–60.0	0.0–0.060	0.0–0.006	167–ND	0.0–0.5
15	12.4	0.013	0.003	333	0.1
	0.0–39.5	0.0–0.040	0.0–0.004	250–ND	0.0–0.3
20	14.9	0.015	0.003	333	0.1
	0.0–42.2	0.0–0.042	0.0–0.004	250–ND	0.0–0.3
Bottom	10.8	0.011	0.002	500	0.1
	0.0–26.7	0.0–0.027	0.0–0.003	333–ND	0.0–0.2

TABLE 3. Summary of Spearman rank correlation coefficients between bacterial production (nmol TdR·m^{-3}·h^{-1}) and various parameters in Hartbeespoort Dam. Only coefficients ≥ 0.4 are given. Number of cases is 30–34 for all depths except bottom (24–28 m) where it is 27–30. Two tailed probabilities are $p \leq 0.001$ for $r \geq 0.5$, $p \leq 0.02$ for $r \geq 0.4$. NS = not significant at $p > 0.05$, S = significant at $p \leq 0.05$ but $r < 0.4$. — = not measured, ND = not detected. EDOC = algal extracellular dissolved organic carbon production (mg C·m^{-3}·h^{-1}), DOC = total dissolved organic carbon (mg·L^{-1}).

Depth (m)	Water temperature	Percent O_2 saturation	Chlorophyll *a*	Phaeopigments	Primary production	EDOC	DOC	Bacterial numbers
0	0.50	0.54	0.81	0.81	0.77	0.53	NS	0.58
0.5	0.73	0.52	0.66	0.45	0.62	NS	—	0.72
1	0.56	0.62	0.76	0.63	NS	NS	—	0.52
3	0.72	0.53	0.63	0.55	−0.40	NS	—	0.75
5	0.55	0.47	0.61	NS	NS	NS	NS	0.54
10	0.47	−0.50	NS	NS	ND	ND	S	0.43
15	0.41	−0.54	NS	NS	ND	ND	S	0.40
20	0.62	−0.66	NS	NS	ND	ND	−0.56	0.51
Bottom	NS	NS	NS	NS	ND	ND	NS	NS

(Fig. 3). Within the euphotic zone bacterial production was correlated to the chlorophyll *a* content (mg·m^{-2})($r = 0.60$, $n = 34$, $p < 0.001$), primary production ($r = 0.56$, $n = 34$, $p < 0.001$) but not with integral EDOC production ($r = 0.23$, $n = 34$, $p > 0.05$).

Water column bacterial production ranged between 43.8 to 1104 nmol TdR·m^{-2}·h^{-1} (Fig. 3), corresponding to 0.3 and 7.0 mg C·m^{-2}·h^{-1}, respectively. As a percentage of integrated primary production, bacterial production for the water column represented 0.1 to 5.5% ($\bar{x} = 0.7\%$), the latter value also being associated with the reservoir overturn event in 1988. Bacterial production of the water column was positively correlated to primary production of the euphotic zone ($r = 0.54$, $n = 34$, $p < 0.001$) and the integrated chlorophyll *a* concentration ($r = 0.49$, $n = 34$, $p < 0.001$) but not with integrated EDOC production ($r = 0.14$, $n = 34$, $p > 0.1$).

Daily values for primary production of the euphotic zone, calculated with Talling's (1965) equation, ranged between 0.44 and 20.1 g C·m^{-2}·d^{-1}. Daily bacterial production rates for the water column were estimated by multiplying the hourly rates by 24 which gave values ranging between 6.7 and 168 mg C·m^{-2}·d^{-1}. Daily bacterial production and primary production were non-linearly correlated ($r = 0.58$, $n = 34$, $p < 0.001$). As a percentage, daily bacterial production represented between 0.3 and 16.8% ($\bar{x} = 2.0\%$) of the daily primary production.

The rates of bacterial protein labelling by leucine and DNA labelling by thymidine co-varied for 10 h in the diel experiment, remaining relatively unchanged during this time (Fig. 4). After 14 h incubation both rates began to increase with the rise in the rate of protein labelling being much sharper than that for DNA labelling. These increased labelling rates coincided with increases in bacterial cell volume and numbers.

Discussion

Relationship between Autotrophic and Bacterial Production

The correlations between bacterial numbers and production, both within and below the euphotic zone of Hartbeespoort Dam

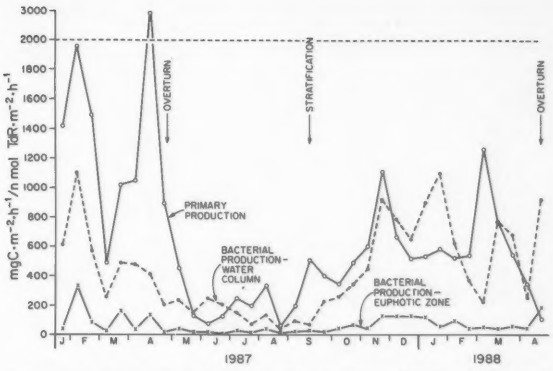

FIG. 3. Areal phytoplankton production (mg $C \cdot m^{-2} \cdot h^{-1}$) and bacterial DNA labeling rate (nmol $TdR \cdot m^{-2} \cdot h^{-1}$) in the euphotic zone of Hartbeespoort Dam. Also shown is the rate of bacterial DNA labeling (nmol $TdR \cdot m^{-2} \cdot h^{-1}$) integrated over the whole water column.

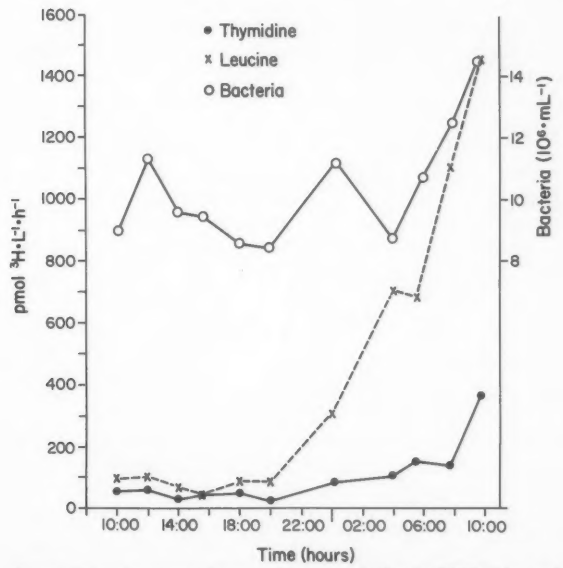

FIG. 4. Diel changes in the rate of protein labeling by [^{3}H]leucine and DNA labeling by [methyl-^{3}H]thymidine in Hartbeespoort Dam bacteria contained in dialysis tubing suspended in the reservoir. Also shown are the changes in bacterial numbers. Data are for 25–26 January 1988.

(Tables 1 and 3), imply a close coupling between autotrophic production of dissolved and particulate organic carbon and the heterotrophic bacterial population numbers and production in this hypertrophic lake. Similar correlations have been found between phytoplankton and bacterial utilization of glucose in the epilimnion of Hartbeespoort Dam (Robarts and Zohary 1986; Robarts 1988; Robarts and Sephton 1988).

Positive correlations between bacterial and phytoplankton production are reported for a diversity of marine and freshwater environments (e.g. Bell and Kuparinen 1984; Lovell and Konopka 1985a, b; Malone et al. 1986; Murray et al. 1986; Riemann and Søndergaard 1986; Scavia et al. 1986; Douglas et al. 1987; Chrzanowski and Hubbard 1988). However, no correlation between bacterial and phytoplankton production was found in the Delaware estuary (Coffin and Sharp 1987) or in the Celtic Sea (Joint and Pomroy 1987); in both studies water temperature was a major factor correlated to bacterial numbers and production. Water temperature was also strongly associated with bacterial number and production in Hartbeespoort Dam (Tables 1 and 3), Lake Arlington, a Texas reservoir (Chrzanowski and Hubbard 1988) and in mesotrophic Lake Biwa, Japan (Nagata 1987). The role of water temperature as a factor regulating the growth of microbial populations has received relatively little attention (Lovell and Konopka 1985c) and demands further investigation, especially the possible interactions with phytoplankton biomass, production, and the supply of organic carbon to bacterial populations (Pomeroy and Wiebe 1988).

In Hartbeespoort Dam the chlorophyll *a* and phaeopigment concentrations and primary production were usually steeply stratified with depth (Figs. 1a and 2). Bacterial numbers were markedly less stratified in their water column distribution (Fig. 1b) while bacterial production was greatest at or near the surface (Fig. 1c, Table 2) in association with large phytoplankton standing stocks and production (Figs. 1a and 2). The decreasing strength of the correlation between bacterial and phytoplankton production with depth (Table 3) suggest an uncoupling of these processes with increasing distance in the water column beneath the euphotic zone.

We found no evidence of a major peak in bacterial production or numbers associated with the aerobic–anaerobic interface or, major declines in these parameters with the presence of the anaerobic zone in Hartbeespoort Dam (Figs. 1b, c). McDonough et al. (1986) found that the rate of [^{3}H]thymidine incorporation into bacterial DNA was greatest in the epilimnion of Lake Oglethorpe, Georgia while the rate of protein labelling was greatest at the aerobic–anaerobic interface. They also found that some obligate anaerobic bacteria in Lake Oglethorpe were capable of assimilating thymidine. Aeration of anaerobic water samples decreased the rate of thymidine incorporation into DNA by 66% (McDonough et al. 1986).

The water column beneath the euphotic zone in Hartbeespoort Dam was, on an areal basis, the major region of bacterial production (Fig. 3). This conclusion is valid even when the morphometry of the reservoir is taken into account. At full supply level ~75% of the volume is contained beneath 3 m, the mean euphotic zone depth. Albright and McCrae (1987) also found that bacterial production per cubic metre was usually greater in the euphotic zone than in deeper aphotic water of a Canadian fjord but when integrated over the water column, approximately 50% of the bacterial production occurred in the deeper aphotic region.

Comparative Bacterial Production Data

The bacterial production (pmol $TdR \cdot L^{-1} \cdot h^{-1}$) of Hartbeespoort Dam is low when compared with that measured in other aquatic environments (Table 4). While the data are not strictly comparable as most were recorded as the rate of thymidine incorporated into cold TCA-precipitable material, which could include labelled macromolecules other than DNA (Robarts et al. 1986), a general trend for increasing bacterial production with increasing trophic status of the system is indicated. This agrees with Cole et al.'s (1988) statistical analysis of bacterial and phytoplankton production data for fresh and marine waters. The highest bacterial production rates were found in nutrient-

TABLE 4. Comparison of bacterial production in some marine and freshwater systems as measured by the rate of incorporation of ^{3}H-thymidine into macromolecules or DNA.

Site	Incorporation rate		Reference
	pmol TdR·L^{-1}·h^{-1}	10^{-21} mol TdR·cell^{-1}·h^{-1}	
Celtic Sea	0.05–5.0	—	Joint and Pomroy 1987
Sargasso Sea, surface	0.09	—	Jonas et al. 1988
Northwest Atlantic, warm core rings	0.5–4.0	0.6–14.7	Ducklow and Hill 1985
Gulf stream rings	0.5–12	1.0–12.0	Ducklow 1986
Atlantic Ocean	1.4–4.7	—	Douglas et al. 1987
Lake Michigan	3.0–15	—	Scavia et al. 1986
Lake Oglethorpe, Georgia	0.0–45	—	McDonough et al. 1986
Lake Biwa, Japan	9.2–115	4.0–18.6	Nagata 1987
Lake Norrviken, Sweden	12.1–205	8.0–155	Bell et al. 1983
Hartbeespoort Dam, South Africa			
surface	1.0–251	0.2–20.0	Present study
bottom	0.0–27	0.0–6.0	Present study
York River estuary, Virginia	42.0–420	8.0–42	Ducklow 1982
Chesapeake Bay, U.S.A.	2.0–635	—	Jonas et al. 1988
Peel-Harvey estuary, Australia	50.0–1555	12.0–124	R. D. Robarts, unpubl. data

enriched estuaries (Table 4) where the entrainment of bottom sediments could have contributed to the high productivities. The data for Hartbeespoort Dam fall at the lower end of the ranges for the estuaries and are similar to those for eutrophic Lake Norrviken. Primary production in Lake Norrviken ranged between 91 and 285 mg C·m^{-2}·h^{-1} (Bell et al. 1983), considerably less than the phytoplankton production of Hartbeespoort Dam (Fig. 3, Robarts 1984). When the bacterial production data in Table 4 were normalized to cell numbers, the Hartbeespoort Dam data were similar to that for significantly less productive systems such as those for the northwest Atlantic rings, Gulf stream rings, and Lake Biwa but lower than for Lake Norrviken, the York River estuary and the Peel-Harvey estuary. These differences probably cannot be ascribed solely to the different methods used as the data for the Peel-Harvey estuary were obtained with the methods used at Hartbeespoort Dam. These data imply, therefore, that although Hartbeespoort Dam is a heavily enriched system, with large phytoplankton standing crops and productivity, bacterial production is low for the degree of autotrophic production and compared with other aquatic environments.

This conclusion is supported by the data on leucine incorporation into bacterial proteins in Hartbeespoort Dam (Fig. 4). During the diel study, the marked increase in the rate of protein labeling followed by the increased rate of DNA labeling and bacterial numbers after about 12 h are characteristic of a container effect (cf. Ferguson et al. 1984) and the shift-up response of bacteria when moving from a balanced to an unbalanced state of growth with substrate enrichment (Kirchman et al. 1986). Prior to this increase the rate of leucine incorporation varied between 50 and 100 pmol·L^{-1}·h^{-1}. These rates are low when compared with the rate of leucine incorporation into bacterial protein measured by Chin-Leo and Kirchman (1988) for Chesapeake Bay, Delaware Bay, and the Mid-Atlantic Bight. Both the covariance of the rates of DNA and protein labeling and their low values relative to other systems support the conclusion that bacterial production is relatively low in Hartbeespoort Dam.

From their analysis of bacterial production in aquatic environments, Cole et al. (1988) presented a series of equations for predicting volumetric and areal rates of bacterial production from chlorophyll *a*, primary production, and bacterial numbers data. For Hartbeespoort Dam the mean surface values of these parameters were 310 mg Chl*a*·m^{-3}, 9190 mg C·m^{-3}·d^{-1}, and 8.83×10^6 cells·mL^{-1} which produced predicted bacterial production values of 122.3, 801.9, and 77.9 mg C·m^{-3}·d^{-1}, respectively. The measured mean was 7.2 mg C·m^{-3}·d^{-1}. Predicted mean areal bacterial production from mean primary production (5322 mg C·m^{-2}·d^{-1}) was 888.5 mg C·m^{-2}·d^{-1} while the measured mean bacterial production rate was 67.5 mg C·m^{-2}·d^{-1}. These calculations support the conclusion that bacterial production was low relative to phytoplankton standing stock and production in Hartbeespoort Dam.

Any extrapolation of bacterial production rates from pmol TdR incorporated into DNA to carbon units is fraught with potential errors because of the large number of untested assumptions and the variability of various conversion factors used (cf. Moriarty 1988; Smits and Riemann 1988). Our bacterial carbon production estimates in Table 2 indicate that bacterial production in Hartbeespoort Dam averaged only 2% of daily, areal primary production. This value is considerably less than the average of 30% calculated by Cole et al. (1988) for fresh and saltwater systems. Even if we had used the conversion factor of 2.14×10^{18} cells·mol^{-1} TdR incorporated as calculated by Smits and Riemann (1988), or other higher conversion factors (e.g. see Moriarty 1988; Smits and Riemann 1988), our overall conclusion would remain unchanged.

It has been demonstrated that bacterial numbers are low relative to the algal standing crop in Hartbeespoort Dam (Robarts 1988). The discrepancy between phytoplankton and bacterial biomasses is even more startling when the small cell size of the bacteria is taken into account. The small cell size of the Hartbeespoort Dam bacteria is generally in line with the decrease in cell volume with increasing trophic status and numbers of bacteria noted by others (Krambeck et al. 1981; Bird and Kalff 1984).

Potential Methodological Errors

A number of potential methodological errors could have led to our underestimating the rate of thymidine incorporation into bacterial DNA. The concentration of exogenously supplied thymidine must be great enough to saturate uptake systems and ensure a constant uptake rate (Moriarty 1986; Chrzanowski

1988). Two methods are available for ensuring that the concentration of TdR is saturating: isotope dilution experiments using labeled and unlabeled thymidine or by adding increased amounts of labeled thymidine at the same specific activity (Moriarty 1986). According to Moriarty, pools of thymidine are rarely, if at all, large (>1 nM) and isotope dilution tends to be more apparent in sediments than in water. Isotope dilution experiments also have weaknesses; for example, if transport across the cell membrane is the rate-limiting step, isotope dilution would still occur but not be measurable (Moriarty 1986).

In Hartbeespoort Dam, the rate of thymidine incorporation into bacterial DNA is linear for at least 2 h (Wicks and Robarts 1987). In the present study we used 30-min incubations in summer and 60-min incubations in winter. Plots of TdR incorporation versus concentration ranging from 1 to 50 nM showed that uptake saturated from as low as 5 nM to as high as 20 nM on different dates. While concentrations in excess of 20 nM did not increase the rate of TdR incorporation, they sometimes caused sharp declines (Robarts et al. 1986). We used 20 nM TdR throughout the present study.

Chrzanowski (1988) reports that although his data for Lake Arlington, Texas showed that the rate of TdR incorporation reached saturation at 10 nM, isotope dilution plots indicated that during summer, when bacterial TdR incorporation was high, dilution occurred and caused underestimates of up to 109%. While the rate of TdR incorporation was significantly underestimated during summer, the seasonal patterns were the same for corrected and uncorrected data.

In light of these experiments it is possible that the low rate of TdR incorporation by bacteria in Hartbeespoort Dam could be ascribed to isotope dilution. However, we added twice the amount of TdR used by Chrzanowski (1988). Further, the major discrepancies in Chrzanowski's data usually occurred at labeling rates of 60 pmol $TdR \cdot L^{-1} \cdot h^{-1}$, or greater, a rate which occurred in Hartbeespoort Dam on only nine dates and mainly in the upper 1 m of the water column (Fig. 1c). In a recent study of intracellular and extracellular isotope dilution in a wide variety of freshwater and marine environments, ranging from oligotrophic to eutrophic, Jeffrey and Paul (1988) found total dilution to be less than the equivalent of 2 nM TdR and concluded that isotope dilution is generally a negligible factor. We conclude TdR dilution was not a major bias in our data.

Another possible reason for the low bacterial production in Hartbeespoort Dam is that not all cells took up and incorporated thymidine. Douglas et al. (1987) found with marine water samples that a greater number of cells incorporated ^{3}H-glutamate than ^{3}H-thymidine. Earlier work in a similar area by Novitsky (1983a) showed that only a small production of both the total and actively growing (Novitsky 1983b) bacteria incorporated thymidine. Pollard and Moriarty (1984) reported that three species of marine pseudomonads did not take up thymidine. Kirchman and Hoch (1988) found that TdR occasionally did not label bacterial DNA in Delaware Bay water samples, similar to our observations in Hartbeespoort Dam (Fig. 1c). The maximum rate of ^{14}C-glucose uptake by bacteria in Hartbeespoort Dam was highly variable, approaching the highest rates published, but more frequently being similar to rates that were characteristic of systems with significantly lower primary production (Robarts 1988). When normalized to bacterial cell numbers the data did not indicate an exceptionally active population analogous to our production data in Table 4. The utilization rate of dissolved free amino acids (DFAA) by bacteria in Hartbeespoort Dam is also low when compared with other systems (Robarts et al. 1990) as is the rate of protein labeling with leucine (Fig. 4). These data do not discount the possibility that some of the bacteria in Hartbeespoort Dam were unable to incorporate thymidine but tends to confirm the low production values recorded.

Non-growing bacteria may have contributed to the low rates of bacterial production measured in Hartbeespoort Dam. On the basis of tests with nalidixic acid, the mean number of metabolically active bacteria at the surface was 20% (range 6.4–48%) of the total population with similar values at 5 and 10 m (Robarts and Sephton 1988). It is not possible to state what impact this had on the rates of bacterial production measured as thymidine incorporation into DNA. Non-growth is a survival mechanism for bacteria subject to starvation (Kjelleberg et al. 1987), a seemingly implausible condition to be found in Hartbeespoort Dam.

Substrate Limitation

On the basis of bacterial cell length averaging 2.15 × cell width in Hartbeespoort Dam, the possibility exists that a protozoan grazer is a major regulator of the bacterial population (see Krambeck 1988). Wright and Coffin's (1984) model of bacterial dynamic interactions with substrate supply and grazing by heterotrophic microflagellates predicts that with high grazing rates, bacterial production approaches the maximum growth rate for the population and bacterial populations will be below the carrying capacity of the system. In the absence of predators the model predicts bacterial growth is a function of the substrate supply. The maximum bacterial population achieved will be representative of the substrate concentration, production will be low and most of the substrate utilized by bacteria will be used for maintenance energy (Coffin and Sharp 1987). On the basis of the low bacterial production rates in Hartbeespoort Dam (Fig. 1c, Table 2), Wright and Coffin's (1984) model would predict that microflagellate grazing was low and that bacterial production was limited by substrate supply.

Robarts and Zohary (1986) have argued that Hartbeespoort Dam bacteria are substrate limited because of the small cell size of the bacteria, their response to an increase in available organic carbon was an increase in metabolic activity per cell followed by an increase in numbers if the substrate concentration remained high, and the glucose concentration, estimated as $(K_t + S_n)$, was usually 10 to 20 $\mu g\ C \cdot L^{-1}$ except at the surface. Our data on the concentration of dissolved free amino acids (measured by HPLC) in Hartbeespoort Dam indicated a range of 1 to 1200 $\mu g\ C \cdot L^{-1}$ with the concentration very frequently being below 10 $\mu g\ C \cdot L^{-1}$ (Robarts et al. 1990). The characteristic bacterial metabolic shift-up response in Fig. 4, and the subsequent increase in bacterial biomass, indicated that the introduction of an available substrate can lead to significant increases in bacterial growth in Hartbeespoort Dam. We conclude, therefore, that the availability of substrate was a major factor responsible for the low bacterial production rates measured in the water column of Hartbeespoort Dam which is in accordance with the importance of biological parameters correlated with bacterial numbers and production in Tables 1 and 3.

Our data for planktonic, heterotrophic bacterial production in this hypertrophic reservoir do not support Bird and Kalff's (1984) hypothesis of an increase in bacterial production per unit bacterial biomass which would maintain a major role for bac-

teria in heavily enriched systems. However, Robarts and Ashton's (1988) mass balance model of organic carbon for Hartbeespoort Dam indicated that the sediments were the major (>95%) site of carbon output, stressing a strong need for bacterial production studies of these sediments.

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Toxicity of Cadmium, Copper, and Mercury to Isolated Trout Hepatocytes

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Freshly isolated rainbow trout (*Oncorhynchus mykiss*) hepatocytes were put in culture and exposed to Cd, Hg, and Cu. Toxicity was evaluated by determining lactate dehydrogenase (LDH) release into the extracellular medium. The cellular response to each individual metal was assessed and the possibility of modulation of Cd effects by either Hg or Cu was tested. Intracellular metal accumulation was measured by radioactive labelling (^{203}Hg) or graphite furnace atomic absorption spectrophotometry (Cd and Cu). It was found that the cells were most sensitive to Cd, while Cu was the least cytotoxic metal; Hg exhibited intermediate activity. The greater cytotoxic potential of Cd over Hg was not attributable to a greater uptake of Cd in comparison to that of Hg. In dual-exposure experiments, Hg had no significant effect on Cd cytotoxicity, but increased sensitivity to Cd appeared when Cu was present. Metal uptake data showed that there was no significant change in the accumulation of Cd due to Cu and vice versa. Trout hepatocytes in culture represent a useful tool for investigating the cytotoxicity of individual metals as well as the effects of environmental metal mixtures.

Des hépatocytes de truite arc-en-ciel (*Oncorhynchus mykiss*) fraîchement isolés ont été placés en milieu de culture et exposés au Cd, au Hg et au Cu. Nous avons évalué la toxicité de ces métaux en déterminant le taux de libération de lactate déshydrogénase (LDH) dans le milieu extracellulaire. Nous avons déterminé la réponse des cellules à chacun de ces métaux et avons vérifié si le Hg ou le Cu pouvait moduler l'effet du Cd. Nous avons mesuré la quantité de métaux incorporée dans les cellules par marquage radioactif (^{203}Hg) ou par spectrophotométrie d'absorption atomique en four de graphite (Cd et Cu). Les cellules manifestaient le plus de sensibilité au Cd tandis que la cytotoxicité du Cu était la plus faible; la cytotoxicité du Hg se situait entre celles du Cd et du Cu. Le fait que le Cd était plus cytotoxique que le Hg n'était pas dû à une incorporation plus importante de Cd dans les cellules. Dans des expériences où les cellules étaient exposées à deux métaux, la présence de Hg n'avait pas d'effet notable sur la cytotoxicité du Cd, mais la présence de Cu augmentait la sensibilité des cellules au Cd. Nos données sur les quantités de métaux incorporées dans les cellules ont montré que la présence de Cu n'avait pas d'effet notable sur le taux d'accumulation de Cd dans les cellules, et vice versa. Les cultures d'hépatocytes de truite sont un bon outil pour étudier la cytotoxicité de différents métaux de même que les effets des mélanges de métaux présents dans l'environnement.

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In Canada, metals represent major aquatic contaminants (Report of the Niagara River Toxics Committee 1984; Hamilton et al. 1987). An important feature of the contamination of aquatic systems by heavy metals is that they are usually present as components of metal mixtures. Despite this situation, the harmful effects of pollutant mixtures (including metals) on aquatic organisms are largely unknown (Hamilton et al. 1987).

The toxicity of metals has been studied in a variety of aquatic organisms and numerous chemical and biological parameters have been examined. Toxicological models have been developed which exploit the advantages of cells in culture (Rachlin and Perlmutter 1968; Marion and Denizeau 1983a, b; Babich et al. 1986). Progress has also recently been made in the isolation and culture of fish hepatocytes, based upon well-established procedures for mamalian hepatocytes (Rauckman and Padilla 1987). Cultured hepatocytes represent a powerful, yet simple, experimental tool for the study of hepatic function in fish, and are particularly well suited for applications in the field of pharmacology and environmental toxicology (Moon et al. 1985).

Research on the effects of heavy metals in trout hepatocytes has recently been initiated in our laboratory (Marion and Denizeau 1988; Gagné et al. 1989). The aim of the present work was to study the cytotoxicity of metal pollutants commonly found in aquatic ecosystems. The model selected uses cultures of rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) hepatocytes because this is a common test species in aquatic toxicology, and because the liver is an important target organ of toxic agents. Cytotoxicity evaluations were performed using lactate dehydrogenase (LDH) leakage into the extracellular medium. The cellular response with each individual metal (Cd, Cu, and Hg) was assessed and the possible molulation of Cd effects by the presence of either Cu or Hg was tested. In addition, intracellular metal accumulation was also determined in an effort to understand the interactions of Hg, Cu, and Cd with trout liver cells.

Materials and Methods

Cell Preparation

Rainbow trout weighing approximately 400 g were obtained from Les Laboratories du Capitaine Bernier, Environnement

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Canada, Longueuil, Québec. The hepatocytes were prepared by a procedure similar to one we used for rat cells (Denizeau et al. 1985) and to that previously described for trout hepatocyte isolation and primary culture (Klaunig et al. 1985). Briefly, the animals were anesthetized with tricaine methanesulfonate (100 mg/L). The abdominal cavity was opened, the portal vein was cannulated, and preperfusion was initiated using calcium-free Hanks' balanced salt solution (HBSS) (Bashor 1979) containing 3 mM EDTA. Preperfusion was maintained until blood was no longer detected in the perfusate. The perfusion was then carried out over a 6- to 7-min period using HBSS supplemented with 60 units/mL collagenase (Sigma type IV). The cells were gently released using a rubber policeman, filtered on a nylon membrane (pore size = 50 μm), and the filtrate centrifuged at 50 × g for 2 min. The pellet was resuspended in Williams' medium E (WME) (Ham and Mckeehan 1979) (Gibco Canada) supplemented with 10% fetal bovine serum (FBS) (Gibco Canada); the samples were then placed in a tube on top of a 2% bovine serum albumin solution in HBSS (HBSA), centrifuged as previously, and the pellets resuspended in HBSA. The cells were counted using a hemocytometer and their viability was estimated from trypan blue exclusion (Patterson 1979). A suspension containing about 2×10^6 viable cells/mL in WME-10% FBS was prepared and distributed in 35-mm Petri dishes (5×10^6 viable cells/dish). The Petri dishes were placed in an incubator and maintained at 15°C and 100% humidity in an atmosphere of 95% air and 5% CO_2.

Cytotoxicity Evaluation

Cytotoxicity was evaluated through the release of lactate dehydrogenase (LDH) into the extracellular medium. Two hours after the cells had been seeded, the medium was removed and three washings were performed using Eagle's minimal essential medium (MEM) (Ham and Mckeehan 1979). The test medium (MEM) supplemented with 1% FBS, 100 units/mL penicillin, 10 μg/mL streptomycin, and containing the metals at the selected concentrations, was added to the cells. The cultures were incubated for 40 h at 15°C under the incubation conditions specified above. At the end of this period, LDH activity in the extracellular medium was determined according to the method of Moldéus et al. (1978). The cells were harvested and centrifuged; an aliquot of the supernatant (100 μL) was mixed with Krebs-Henseleit buffer, pH 7.4 (900 μL), containing an excess of pyruvate and NADH. The initial rate of NADH oxidation was obtained by the decrease in absorbance at 340 nm with time, over the period (about 5 min) when the relationship between the two parameters was linear. Enzymatic activity was expressed in units per litre (U/L). At the concentrations used, the metals studied did not affect LDH activity.

Cellular Accumulation of Metals

The hepatocytes were exposed to the test medium for 40 h at 15°C, as performed in cytotoxicity experiments, except that cold Hg was replaced by ^{203}Hg ($^{203}HgCl_2$, New England Nuclear, specific activity: 0.5 Ci/mmole). At the end of the incubation period, the cells were washed twice by centrifugation in MEM. The pellet was resuspended in HBSS. Half of the suspension was treated with NaOH (0.3 N final concentration) at 37°C for 1 h. Aliquots were used either for protein determination (Bradford 1976) or for scintillation counting when ^{203}Hg was present. The other half of the suspension was processed for cold metal (Cd and Cu) analysis in the following manner: concentrated nitric acid (Baker Instra-Analyzed) was added (2 N final concentration) to the samples; digestions were carried out at 60°C for 76 h; metal measurements were performed by atomic absorption spectrophotometry using a Varian GTA-95 graphite furnace and a Varian AA-1475 spectrophotometer.

Preparation of Test Media

Metal stock solutions, which were diluted with culture media to the appropriate final concentrations, were prepared according to the procedures of the Association of Official Analytical Chemists (AOAC 1984). Cadmium, Hg, and Cu were in the form of $Cd(NO_3)_2$, $HgCl_2$, and $CuSO_4$, respectively. The stability of the test media with respect to metal solubility was verified in the following manner: immediately prior to using the test medium in the cultures, aliquots were taken for metal analysis as previously described. In all cases it was found that at least 99% of the metal was present in the liquid phase.

Analysis of the Data

Statistical analysis was performed on paired groups (untreated versus single-exposure and single-exposure versus dual-exposure groups) using Student's *t*-test.

Results

A first series of experiments was conducted to establish the concentration–response profile for each individual metal. Hepatocytes were exposed to increasing concentrations of either Cd, Hg, or Cu. At lower levels (<0.2 μM Cd, <1.0 μM Hg, and <20 μM Cu), cytotoxicity was not apparent, as judged from the release of LDH into the extracellular medium (Fig. 1). Maximum observed loss of cellular enzyme occurred at 2 μM Cd, 25 μM Hg, and 800 μM Cu. The data suggest that trout hepatocytes are most sensitive to Cd. Furthermore, Hg exhibits less potency than Cd. In contrast, Cu is clearly the least cytotoxic of the three metals.

As far as the two nonessential metals Cd and Hg (which are not detectable in untreated cells) are concerned, the difference in potency is apparently not attributable to a greater accumulation of Cd in comparison to that of Hg. Indeed, at similar low exposure concentrations (Fig. 2), intracellular Cd and Hg levels were comparable. However, at 0.9 μM, the threshold for Cd cytotoxicity was less than that of Hg which occurs at levels significantly above 1 μM (Figs. 1A and B).

The second series of experiments tested the possibility of modulation of Cd cytotoxicity by Hg or Cu. Concentrations for the combined exposures were selected in conformity with a model which has already been applied to the study of mixture toxicity, and according to which, one of the substances is considered inactive when given alone (Mitchell 1976). In the experiments on combination of Cd and Hg, Hg was used at a concentration which, when alone, had no statistically significant effect (0.5, 1.0 μM Hg) while Cd was present at a concentration which induced a detectable response. Although in one instance the release of LDH appeared to be slightly stimulated by Hg (0.5 μM Hg and 0.45 μM Cd), there was no overall tendency suggesting that Hg had a significant effect on Cd cytotoxicity (Fig. 3). Metal accumulation was determined under the same conditions. The amount of Cd taken up by the hepatocytes was reduced to some extent when Hg was added to the cultures, although this was not statistically significant

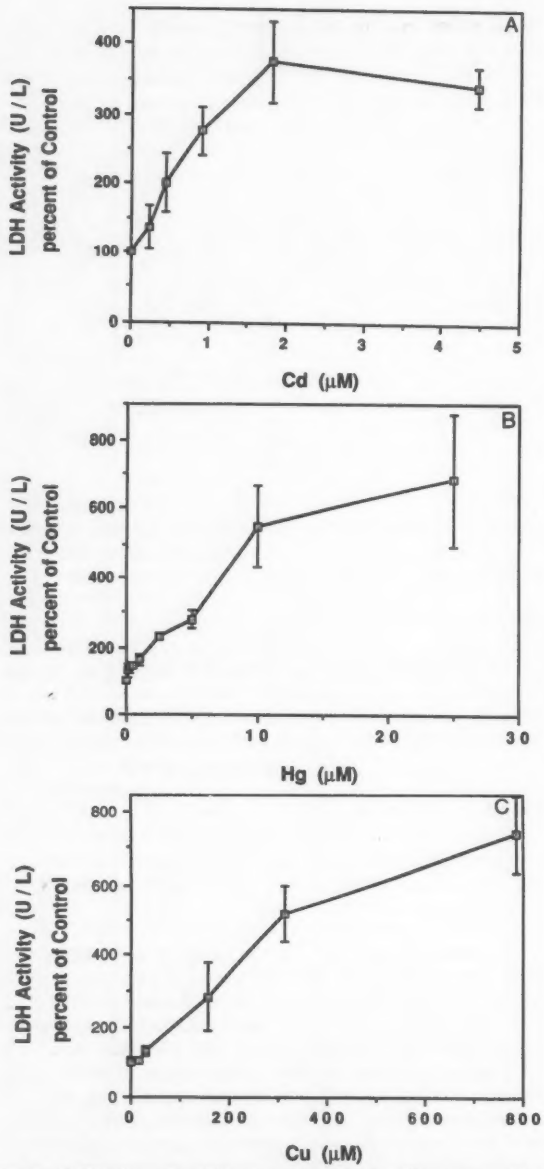

FIG. 1. LDH release in rainbow trout hepatocyte cultures exposed to heavy metals. (A) Cd, (B) Hg, and (C) Cu. The incubation time was 40 h at 15°C. The data represent means ± SD for six determinations (two experiments of sample size $n=3$) and are expressed in enzyme units per litre (U/L). In the controls, the levels of activity were of 238 ± 22, 187 ± 36, and 149 ± 52 U/L for the series of experiments involving Cd, Hg, and Cu, respectively.

(Fig. 4). Furthermore, intracellular Hg levels were unchanged in the presence of Cd (data not shown). The data suggest some Hg/Cd additivity of toxic effect.

The other mixture examined consisted of Cd and Cu. Copper at 7.9 and 15.7 μM did not cause any increase in extracellular enzyme activity when alone (Fig. 1C). When combinations were tested, mean values obtained for three separate experiments

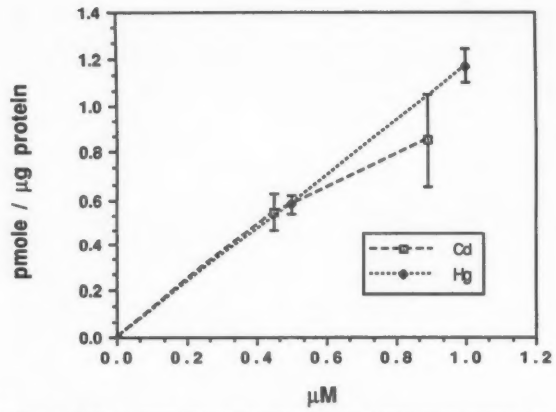

FIG. 2. Metal accumulation by rainbow trout hepatocytes exposed to heavy metals. The data represent means ± SE for three experiments of sample size $n=3$.

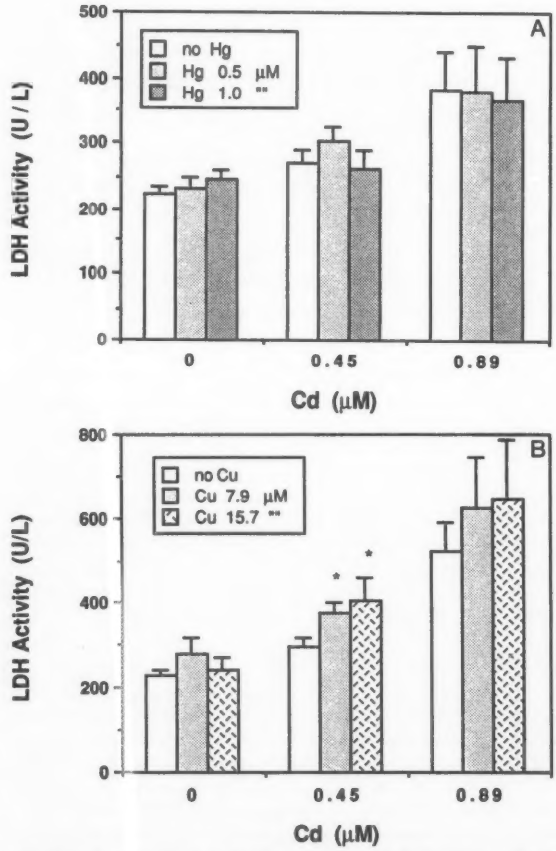

FIG. 3. Cytotoxic effects of Cd in the presence of Hg or Cu. (A) LDH release in the presence of Cd and Hg, (B) LDH release in the presence of Cd and Cu. Means ± SE for three experiments of sample size $n=3$ are represented. *$P<0.05$.

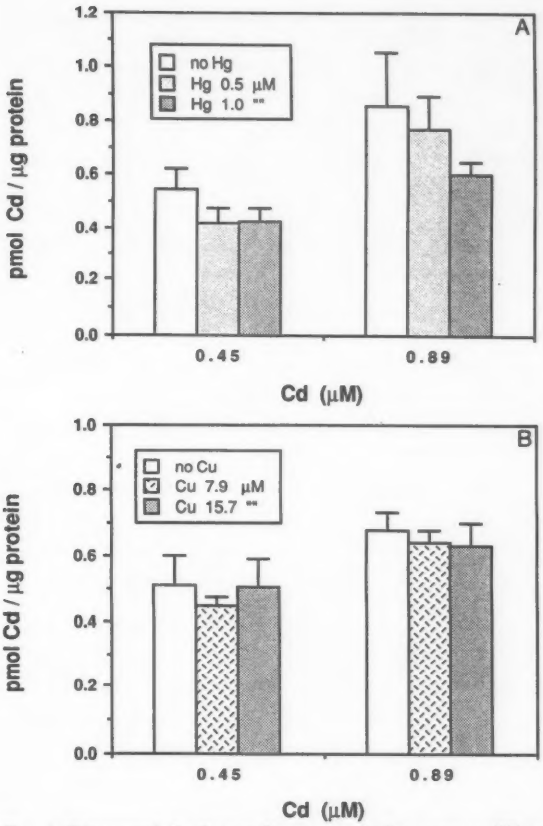

FIG. 4. Cd accumulation in trout hepatocytes in the presence of Hg or Cu. (A) Cd accumulation in the presence of Hg, (B) Cd accumulation in the presence of Cu. Means ± SE for three experiments of sample size $n = 3$ are represented.

showed an increased sensitivity to Cd when Cu was present (Fig. 3B): the effects of the two treatments applied together appeared greater than those which could be predicted from the response of the separate treatments. This was statistically significant (Student's t-test) at combinations involving 0.45 µM Cd. Intracellular metal levels were also determined but there was no significant change in the accumulation of Cd due to addition of Cu (Fig. 4B). Similarly, intracellular Cu was unaffected by Cd at the concentrations tested (data not shown). These observations suggest that Cd cytotoxic effects can be modulated by Cu in a synergistic fashion.

Discussion

Rodent hepatocyte cultures have been widely used as models for toxicological evaluations. Cytotoxicity, xenobiotic metabolism, detection of carcinogenic potential, and molecular mechanisms of action of toxic agents have all been successfully investigated with mammalian hepatocytes (Rauckman and Padilla 1987). More recently, interest has developed concerning the use of isolated fish hepatocytes, following the example of usefulness of homologous mammalin cell cultures. It has been demonstrated that not only could fish hepatocytes be isolated with satisfactory yields and viability, but that they could also be adequately maintained in primary culture for several days (Klaunig et al. 1985). A number of reports on the use of trout hepatocytes and focusing on culture methodology (Lipsky et al. 1986; Kocal et al. 1988), on the study of hepatic function (Walton and Cowey 1979; Hazel and Prosser 1979; French et al. 1981; Maître et al. 1986), or on the investigation of xenobiotic metabolism (Parker et al. 1981; Walton et al. 1984; Loveland et al. 1987) demonstrate the potential of cell culture studies.

The quality of cell preparations obtained from trout liver has been assessed using the usual criteria applied to mammalian hepatocytes. For example, the integrity of the cell membrane has been evaluated through the exclusion of vital dyes and leakage of intracellular enzymes such as LDH (Walton and Cowey 1979; Klaunig et al. 1985). Because the release of LDH has proven to be a simple and reliable indicator of cytotoxicity which is well correlated with the loss of cell viability in hepatocytes (Tyson and Green 1987), this parameter was chosen to initiate the study on the effects of heavy metals on trout liver cells.

Representative environmental metal contaminants were selected in undertaking the study of their effects on fish liver cells in vitro. The utility of such an approach is well supported by the fact that the liver is a site of accumulation of Cd, Hg, and other metals in rainbow trout. For example, liver Cd of adult rainbow trout exposed to 3.6 µg Cd/L for 178 d increases 250- to 400-fold over the test period (Giles 1988). Similarly, fishes subjected to a sub-lethal concentration of Cd (9 µg/L) for 14 d, exhibit a Cd liver content of 0.26 µg/g dry weight (Roberts et al. 1979). In juvenile rainbow trout exposed to 100 µg/L Hg (as $HgCl_2$) in the water for 14 d, Hg is found in the liver, within the hepatocyte lysosomes (Baatrup et al. 1986). Alterations of biochemical and physiological parameters in the liver of rainbow trout exposed to Cd and Cu have also been described (Arillo et al. 1984).

There has been a limited number of studies which focused on the toxicity of heavy metals to rainbow trout cells in vitro. In these few investigations, a well established cell line (RTG-2) has been used (Marion and Denizeau 1983b). In RTG-2 cells, 1 µM Cd caused detectable cytotoxic effects (inhibition of [^{14}C] uridine incorporation into RNA) only after 6 d of exposure. The present data show significant cytotoxicity of Cd to hepatocytes at a similar concentration and for an incubation period of 40 h (Fig. 1A). In both series of experiments, the culture medium was the same (MEM supplemented with 1% FBS). Although the parameters used to detect cytotoxicity were different, it appears that hepatocytes in primary cultures are more sensitive than the gonad-derived cell line (RTG-2). Furthermore, based upon the concentrations that reduced the uptake of neutral red by 50% following a 24-h incubation with the test metal, Cd was found to be more cytotoxic than Cu to RTG-2 cells (Babich et al. 1986). These toxic concentrations were of 0.18 and 1.45 mM for Cd and Cu, respectively. Again, these values support the view that the RTG-2 trout cell line is less sensitive than trout hepatocytes in primary culture.

Investigating the combined toxicity of environmental contaminants is a difficult task. In aquatic ecosystems, for example, heavy metals occur simultaneously and organisms are subjected to multiple exposures. A classical strategy to tackle the problem of mixture toxicity consists, at the initial stage of investigation, of focusing on two compounds at a time (National Research Council 1980). In the present study, preliminary data have been obtained concerning the response of hepatocytes exposed to two heavy metals. A first series of results with Cd

and Hg do not allow us to conclude on the one hand that Cd toxicity is modulated by Hg (Fig. 3A), even though there was a slight but not statistically significant reduction of Cd accumulation in the presence of Hg (Fig. 4A). On the other hand, another series of observations suggest that Cu could enhance Cd cytotoxic effects (Fig. 3B), without affecting its accumulation by the hepatocytes (Fig. 4B). In the light of the present data, it appears that further work is necessary to better understand the mechanisms of cytotoxicity of heavy metal mixtures on trout liver cells. For example, studies including the elucidation of the role of metallothionein (MT), a specific metal-binding protein, in response to heavy metal intoxication are especially required. This is of particular interest since we have recently been able to show that Cd (Gagné et al. 1989) and Hg (F. Denizeau and M. Marion, unpubl. data) induce MT synthesis in primary cultures of trout hepatocytes.

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Thin Layer Interference may Reduce the Visibility of Transparent Phantom Midge Larvae (*Chaoborus trivittatus*) to Predators

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Giguère, L. A., and R. L. Dunbrack, 1990. Thin layer interference may reduce the visibility of transparent phantom midge larvae (*Chaoborus trivittatus*) to predators. Can. J. Fish. Aquat. Sci. 47: 1043–1046.

The body wall of transparent aquatic animals is constructed of materials which have a higher refractive index than water. Because of this, light will reflect from their surface, making their body contours visible to predators. Electron micrographs of cuticle from the transparent pelagic stage of the phantom midge *Chaoborus trivittatus* reveal thin layers less than a wavelength of light thick which could alter the spectral composition of this surface reflection by acting as an interference filter. This hypothesis is supported by measurements of the reflectance of the body surface of *C. trivittatus*. Reflectance was lower than expected at the wavelengths which maximize contrast in aquatic environments and higher than expected at ambient background wavelengths, suggesting that interference may reduce the visibility of *Chaoborus* to its predators by improving the spectral matching of its body reflection to the visual background.

La paroi du corps des animaux aquatiques transparents est formée de matières dont l'indice de réfraction est plus élevé que celui de l'eau. C'est pourquoi la lumière se reflète sur ces animaux et rend les contours de leur corps visibles pour les prédateurs. Des micrographies électroniques du cuticule du moucheron *Chaoborus trivittatus* à son stade pélagique transparent révèlent la présence de fines couches de moins d'une longueur d'onde lumineuse d'épaisseur qui pourraient modifier la composition spectrale de cette réflexion superficielle en agissant comme filtre d'interférence. Cette hypothèse est appuyée par des mesures de la réflectance de la surface corporelle de *C. trivittatus*. La réflectance était inférieure à ce que l'on s'attendait aux longueurs d'ondes qui maximisent le contraste dans des environnements aquatiques et supérieure aux longueurs d'ondes naturelles ambiantes, ce qui laisse croire que l'interférence peut réduire la visibilité de *Chaoborus* pour ses prédateurs en améliorant la coïncidence spectrale de sa réflexion corporelle au fond visuel.

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Transparency is a tactic used by many zooplankters to avoid detection by visually foraging fishes. Transparency is never perfect, however, and despite the substantial reduction in visibility that accompanies transparency, transparent organisms are an important dietary item for many aquatic predators. The visual cues that allow predators to locate transparent prey are provided by the contrast between the ambient visual background and the light which reflects from the interface between the water and the prey's more refractive integument. Under daylight conditions the differences in the quality of these light sources, which are responsible for visual contrast, can be substantial. This is particularly true in shallow water where prey illuminated predominantly by bright downwelling light (Easter 1975; Lythgoe 1975) may be viewed against a background of horizontally transmitted sidewelling light, which is less intense and more likely to be dominated by light of shorter wavelengths (Tyler and Smith 1970; Easter 1975; Lythgoe 1975; McFarlane and Munz 1975a). These differences in spectral composition between sidewelling and downwelling light are a result of the higher rate of attenuation of long wavelength light. Sidewelling light arriving at some point will have travelled further in the water column, on average, than the corresponding downwelling light reaching the same point, and as a result will have lost relatively more of its longwave component. Therefore a transparent prey which reflects downwelling light horizontally toward a nearby predator will appear relatively redder to that predator than the visual background, and the visual contrast of the prey (i.e. the ratio of the brightness of light reflected from the prey's surface to that of the visual background) will consequently be greatest for long wavelength light. The marked longwave sensitivity which characterizes the visual systems of epipelagic and shallow water fishes, particularly those from freshwater (McFarland and Munz 1975b; Hailman 1977; Easter 1975), suggests that this spectral dependence of visual contrast may have played an important role in the evolution of predator–prey interactions in aquatic communities (McFarlane and Munz 1975c).

Although the sensitivity of the visual systems of aquatic predators may be adapted to exploit spectral differences between sidewelling light and reflected downwelling light, selection on their prey organisms in turn will have favoured the evolution of behavioural or structural counter-measures which alter the

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spectral composition of this reflected light to better conform to the blue-dominated visual background. One method of achieving this spectral alteration which could be particularly useful for transparent organisms is thin layer interference, a phenomenon commonly employed by aquatic and terrestrial animals to produce iridescent body coloration (Denton and Nicol 1965a, b; Denton and Land 1967; Fox 1976; Lythgoe 1979).

Thin layer interference is usually associated with systems of partially reflecting surfaces which have inter-surface spacings on the order of a single wavelength of visible light. In the simplest case, interference arises when coherent (i.e. in phase) light travelling through some medium is transmitted through one interface, reflects from the next, and then interferes, as it is retransmitted through the first interface, with the light which is reflected from that interface back into the original medium. Destructive interference, which reduces reflection from an interface, is maximized when the phase difference between these interfering sources is one-half wavelength. Reflection enhancing constructive interference is maximized when this phase difference is a full wavelength (i.e. the sources are in phase). Because the visible spectrum spans the wavelength range of approximately 400/N nm to 700/N nm, where N is the refractive index of the medium, destructive interference for one visible wavelength may be associated with constructive interference for others. For example, a 350/N to 400/N nm phase difference, which would result in destructive interference for red light (700/N nm), is a constructive phase difference for blue light (400/N nm). Such a phase difference produced by thin layers in the body wall of a transparent zooplankter could thus enhance shortwave reflectance and reduce longwave reflectance. This would alter the spectral composition of the downwelling light reflected from its body wall to better match the blue dominated background, thereby reducing its visibility to predators.

On the basis of evidence that the integument of insects seems to be readily adaptable for the production of optical interference (Neville 1975), we looked for evidence of a thin layer interference system in the body wall of the freshwater larvae of the phantom midge *Chaoborus trivittatus*. These transparent, pelagic larvae require up to 2 yr to achieve maximum lengths of approximately 15 mm. Transparency contributes significantly to their ability to survive the long period of exposure to aquatic predators required to attain this unusually large size for a zooplankter. The effectiveness of their transparency is underlined by studies which show that the probability of *Chaoborus* being detected by predatory fish is significantly increased when their transparency is reduced by the presence of a visible food bolus in their crop (Giguère and Northcote 1987).

Materials and Methods

The spectral reflectivity of the body wall of fourth instar *Chaoborus* larvae was determined as follows: live larvae were exposed to white light for 48 h and then fixed in 4% formaldehyde. A sample of fixed integument was placed on a glass slide with a drop of water and reflectance measurements were then made at 30-nm intervals over the wavelength range (in air) of 400 to 700 nm using a Zeiss Photomicroscope III+ (photomultiplier HTV 446).

Transmission electron micrographs of the integument of fourth instar *C. trivittatus* were examined for the existence of thin layers. Body wall samples from *Chaoborus* (48-h white light exposure, as above) were fixed for 5 h at 24°C with 3% glutaraldehyde and postfixed for 2 h in 2% OsO_4 in a cacodylate buffer (pH 8.2). Samples were dehydrated in ethanol and treated in propylene oxide before embedding in Spurr's epon. Transverse sections cut from the imbedded material were stained with lead citrate.

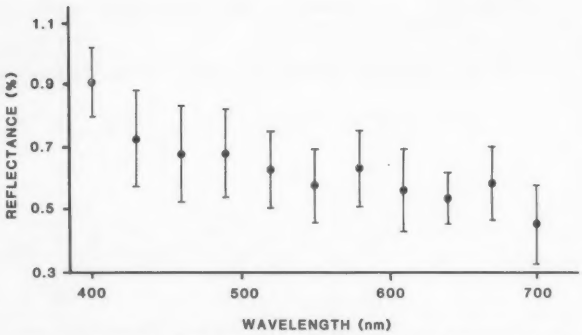

FIG. 1. Spectral reflectance measurements from the integument of fourth instar *Chaoborus trivittatus*. Means are for two measurements on each of four individuals. Error bars represent 95% confidence intervals. The measurements were taken relative to a vitrinite standard of refractive index 1.69446. Expected reflectance of cuticle, estimated from Fresnel's equation for normal incidence, is 0.72%.

Results and Discussion

Reflectance of normally incident light from the body wall of *Chaoborus* declined from a mean value of 0.91% at 400 nm to 0.55% at 700 nm (Fig. 1). The predicted value for insect cuticle (refractive index approximately 1.58) obtained from Fresnel's equation is 0.72%; thus the reflectance of the body wall of *Chaoborus* is lower than expected for long wavelengths (red) and higher than expected for short wavelengths (blue), a trend which is statistically significant (ANOVA; $F_{10,70} = 13.88$; $p \ll 0.01$). Although all reflectance measurements were less than 1%, these measurements are for normally incident light and, because reflectance increases with the angle of incidence, therefore represent minimal values. Reflection is predicted to rise to 3% at 45°, and approach 100% at grazing incidence. The potential significance of these reflectance values becomes more apparent when they are compared with the radiance of the sidewelling background light. Approximately 3% of vertically transmitted downwelling light incident at 45° on the body wall of a cylindrical zooplankter oriented with its long axis in the horizontal plane, will be reflected horizontally. However, because the radiance of vertical downwelling light may be 10–100 times greater than that of horizontal light during daylight hours (Tyler and Preisendorfer 1962; Lythgoe 1975), the overall brightness of the reflected light may be comparable with the brightness of the visual background, particularly for longer wavelengths. Hence the contrast produced by this reflection could contribute substantially to prey visibility.

Electron micrographs of the body wall of fourth instar *Chaoborus* larvae revealed the existence of thin layers. In general, a thin dark outer layer was followed by 10 to 30 layers each consisting of a light and a dark band (Fig. 2). It was assumed that the influence of interference on the reflectance of the body surface would be primarily a consequence of the spacing of the outermost layers. Therefore mean layer thickness was estimated as follows: for each of five larvae, four electron micrographs

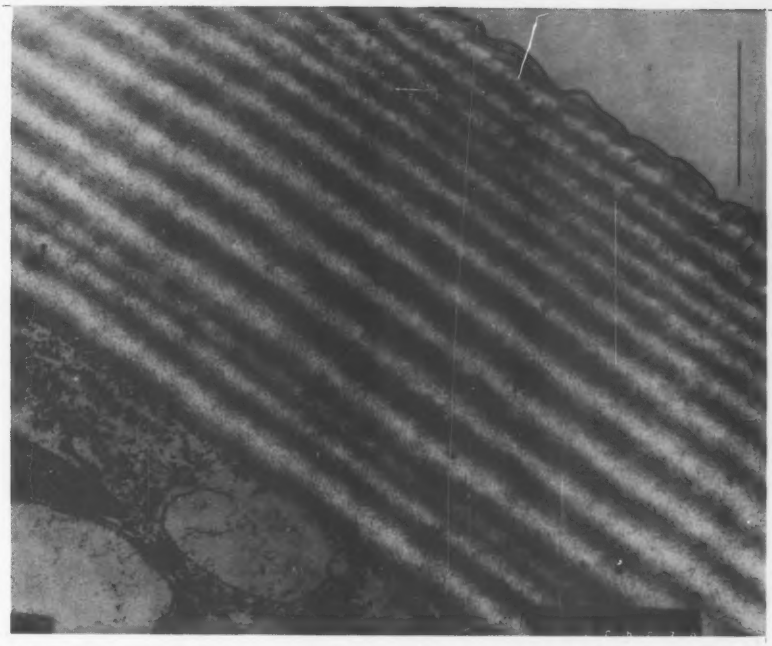

FIG. 2. Electron micrograph of a transverse section of the integument of a fourth instar *Chaoborous trivittatus*. Note the faint parabolic patterns, indicative of helicoidal cuticle (see text), visible within the larger light internal bands. Layer thickness (one light plus one dark band) was measured for the three bands adjacent to the outer body surface (see in lower right hand side of photograph). Scale bar represents 1000 nm.

were obtained representing cross-sections from the dorsal, ventral, right lateral, and left lateral body wall regions. The total width of the first three layers was then measured directly from the electron micrograph prints with calipers at three equidistant points along the body wall circumference in each micrograph. These measurements were then converted into mean layer thickness (three per photomicrograph print). There was no significant difference in mean layer thickness between lateral, dorsal, or ventral sections (ANOVA). Therefore the three means for each of the 20 prints were pooled. Overall mean layer thickness was 153 nm (SD = 23; $n = 60$).

Both the reflectance and microscopy results are consistent with the presence of thin layers in the body wall of *C. trivittatus* capable of altering the spectral composition of reflected light. To determine whether the mean layer thickness given above accords with the observed pattern of reflectance, we compared our data with the currently accepted model for the structure of cuticle layers in arthropods (Bouligand 1972). According to this model, the arthropod cuticle consists of birefringent chitin crystallites imbedded in a protein matrix. These crystallites are arranged in very thin sheets within which the crystallites are parallel both to each other and to the body surface. Under certain conditions each crystallite sheet may be rotated at a constant angle with respect to that immediately below, with the successive rotation of these sheets forming a helical stack with the axis of the helix perpendicular to the body surface. The helical rotation of these chitin sheets produces alternating light and dark bands when the cuticle is viewed in cross-section with the transmission electron microscope. If these cross-sections are slightly off the vertical, characteristic parabolic patterns can be seen within the bands. Electron micrographs of the cuticle of *C. trivittatus* reveal alternating light and dark bands as well as the diagnostic parabolic patterning (Fig. 2); both are indicative of a helicoidal structure. A 180° rotation about the axis of the helicoidal stack of crystallite sheets corresponds morphologically to the distance from a position on a light or dark band to the corresponding position on the next band of the same shade (e.g. one light plus one dark band) (Neville and Caveney 1969). Neville and Caveney (1969) provide evidence that the intrinsic birefringence of the chitin crystallites combined with this helical arrangement produces an effect analogous to that of a series of optically identical partially reflecting surfaces spaced a distance S apart, where S is the distance for a 180° turn of the helix (e.g. one light plus one dark band; see above). Surfaces arranged in this manner will give rise to optical interference and, because of this, will have the property of selective reflection. For normally incident light the wavelength, ϕ, most strongly reflected for such a system will be that for which the optical distance between adjacent reflecting surfaces is 1/2 ϕ, i.e.

$$(1) \quad \phi = 2 \cdot S \cdot N,$$

where N is the refractive index of cuticle (Neville and Caveney 1969). A reflectance minimum due to destructive interference would occur at the wavelength, Ω, for which the optical distance between surfaces is 1/4 Ω.

From our results $S = 153$ nm, $N = 1.58$, and hence $\phi = 483$ nm. This result is in general agreement with our peak reflectance of approximately 400 nm. Reflectance should decrease continuously throughout the visible spectrum to a min-

imum outside visible range at $\Omega = 966$ nm). This is also in qualitative agreement with Fig. 1. For light at angles of incidence other than normal, equation (1) is modified to

$$(2) \quad \phi = 2 \cdot S \cdot N \cdot \cos \theta,$$

where θ is the angle of incidence measured from the vertical. The reflectance curve is therefore predicted to shift toward shorter wavelengths as the angle of incidence increases.

Although body wall layers similar to those we have observed in *C. trivittatus* are not uncommon in the arthropods, there is substantial variation in the thickness of these layers, and hence in their optical properties, both between species and between areas within individuals (Neville 1975). The thickness of these layers, and hence the quality of light reflected from them, would therefore appear to be a property of chitinous exoskeletons which is subject to modification by selection. We have evidence that the spacing of these layers may also be under behavioural control. In *C. trivittatus* reflectance and layer spacing were found to vary with both the intensity and spectral composition of ambient light (48-h exposure prior to fixing; L. A. Giguère and R. L. Dunbrack, unpubl. data).

In summary, the results presented here provide evidence that thin layers within the chitinous body wall of *C. trivittatus* adaptively alter the spectral composition of reflected light in such a way as to reduce mortality due to visual predation. Such adaptations may not be restricted to *Chaoborus* however. We have also observed thin body wall layers in a number of other aquatic species including crustaceans, larval fishes, and chaetognaths (L. A. Giguère and R. L. Dunbrack, unpubl. data). Although the spacing of layers in these taxa is comparable with that found in *Chaoborus*, it is presently not known if they function as interference filters. However, their presence suggests that the use of thin layer interference may be widespread amongst transparent zooplankton, and hence a significant factor in trophic interactions within aquatic communities.

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Phytoplankton in Quebec Lakes: Variation with Lake Morphometry, and with Natural and Anthropogenic Acidification[1]

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Phytoplankton of 54 Québec lakes were studied to determine the effects of acidification, physico-chemistry, and lake morphometry on the community composition. The phytoplankton groups data matrix was submitted to a flexible linkage cluster analysis. The resulted six clusters of lakes are distributed along the northeast to southwest geographic axis and are related to an ascending density gradient in total phytoplankton, chlorophytes, cyanophytes, and microflagellates. Factor analysis produced five derived environmental factors (hardness-alkalinity, lake depth and transparency, dystrophy, lake size and salinity-sulphates loading), which explain 73% of the total variance of the abiotic data matrix. Alkalinity–acidity gradient is the most important factor related to phytoplankton groups and species distribution. Most of the cyanophytes taxa and some chlorophytes are positively related to the hardness-alkalinity and the sulphates loading factors. The abundances of pyrrophytes increases in alkaline lakes subjected to great sulphates loading. Lake morphometry and trophy also influence the phytoplankton distribution. Deep and clear lakes in eastern Quebec are dominated by chrysophycean species. Few species are related to the dystrophy factor. Phytoplankton distribution and relationships with environmental factors in Quebec lakes are compared with studies of the phytoplankton composition in Newfoundland and Labrador lakes.

Le phytoplancton de 54 lacs du Québec a été analysé pour évaluer les effets de l'acidification, de la physico-chimie et de la morphométrie des lacs sur la composition de cette communauté. Une analyse de groupement à liens flexibles a été appliquée à la matrice des abondances des groupes de phytoplancton. Les six groupements de lacs produits se distribuent selon l'axe géographique nord-est à sud-ouest en fonction d'un gradient ascendant d'abondance du phytoplancton total, des Chlorophytes, des Cyanophytes et des Microflagellés. L'analyse factorielle a produit cinq facteurs environnementaux composites (dureté–alcalinité, profondeur et transparence des lacs, dystrophie, taille des lacs, apports en sels et en sulfates) qui expliquent 73% de la variance totale de la matrice des variables abiotiques. Le gradient alcalinité-acidité représente le principal facteur relié à la distribution des groupes et des espèces de phytoplancton. La plupart des Cyanophytes et certains Chlorophytes présentent des corrélations positives avec les facteurs dureté-alcalinité et apports en sels et en sulfates. L'abondance des Pyrrophytes est plus élevée dans les lacs alcalins ayant des apports en sulfates importants. La morphométrie et la trophie des lacs influencent aussi la distribution du phytoplancton. Les lacs profonds et clairs du nord-est du Québec sont dominés par les Chrysophytes. Peu d'espèces sont reliées au facteur de dystrophie. La distribution du phytoplancton et les relations avec les facteurs environnementaux dans les lacs du Québec, sont comparées aux études de la composition du phytoplancton dans les lacs de Terre-Neuve et du Labrador.

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Our understanding of how acidification and other environmental factors influence phytoplankton composition in lakes is limited. According to Stokes' (1986) recent review, the responses of primary producers to acidification should be considered in terms of community responses (taxonomic composition, biomass, and functional aspects), individual species responses to lowered pH, and proposed or demonstrated mechanisms to explain community changes (Schindler et al. 1985). To infer plankton changes induced by acidification, most research has involved comparisons between acid and neutral lakes or comparisons among lakes of different pH (Bleiwas et al. 1984; Brezonik et al. 1984), assuming that lakes were very similar for other physical, chemical, and trophic features within the same geographic and climatic regimes. It seems likely that factors other than pH are clearly related to phytoplankton responses. Lake morphometry and drainage basin area have an important influence on lake trophic state and pronounced effects on phytoplankton abundance, structure, and production (Schindler 1971; Pridmore et al. 1985; Earle et al.

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1986, 1987). Nutrient loading (Persson et al. 1977; Pick and Lean 1987), ratio of nitrogen to phosphorous (Smith 1982), and dissolved humic matter (Eloranta 1986) may be of importance as well.

A limnological and fisheries survey of 54 Quebec lakes was carried out in 1982 by Department of Fisheries and Oceans Canada (Langlois et al. 1983), with the purpose of obtaining a reference source of information on acid precipitation effects on Quebec lacustrine ecosystems. This survey, which included phytoplankton data, made it possible to investigate relationships among phytoplankton communities and the physical and chemical environment of Quebec lakes, in reference to both lake morphometry and, natural and anthropogenic acidification. In Quebec, earlier investigations concentrated either on taxonomic aspects or ecological works but a comprehensive study of the phytoplankton structure in relation to lake's abiotic environment is still lacking. Early taxonomic works were carried out by Venne (1967); and Plinski and Brunel (1977). Contant and Duthie (1978) published an annotated list of the phytoplankton species of Lake St-Jean. Reports on phytoplankton abundance, succession, seasonality, primary production, and diurnal migration in Quebec lakes and rivers have been published by Alaerts-Smeesters and Magnin (1974); Plinski and Magnin (1975, 1979); Janus and Duthie (1979a, b); Ross and Duthie (1981); Dumouchel and Magnin (1982); Hamilton and Duthie (1987); and Chow-Fraser and Duthie (1987). Recently, seasonal variations in composition and size structure of the phytoplankton community in a small laurentian Shield lake has been investigated by Jordan et al. (1988).

The purpose of this study is to define the general structure of the phytoplankton communities within Quebec lakes and then to describe the relationships between the critical environmental factors and the dominance of phytoplankton groups or species. New ecosystem approaches using multivariable statistics, factor and correlation analyses are becoming commonly used in studies on the effects of abiotic factors on aquatic communities (Allen et al. 1977; Green and Vascotto 1978; Pinel-Alloul et al. 1979, 1982, 1987; Brezonik et al. 1984; Earle et al. 1986, 1987). In this paper, the use of multivariable statistics summarizing the variability of a complex data set of environmental factors enables us to determine the relative effects of acidification, physico-chemistry, and lake morphometry on the abundances of phytoplankton groups and species.

Methods

Study Area

Fifty-four lakes were sampled throughout Quebec (Fig. 1) for a suite of morphometric, physical, chemical, and biotic variables (Langlois et al. 1983). The general features of study lakes were already presented by Pinel-Alloul et al. (1990) in a study of zooplankton communities in the same lake sample. The study area is considered very sensitive to acidification (Bobée and Lachance 1982; Dupont and Grimard 1986). A southwest to northeast geographic gradient in H^+ ions concentration and an inverse gradient in the sulphate concentrations have been detected in the lake sample (Lachance et al. 1984; Pinel-Alloul and Méthot 1985; Pinel-Alloul et al. 1987). Furthermore, a recent study (Department of Fisheries and Oceans 1987) showed that 72% of the lakes exhibited alkalinity of less than 100 $\mu eq \cdot L^{-1}$ and had alkalinity/sulphate ratios below 1. Both parameters indicate an extreme sensibility to acidification (Bobée et al. 1982).

Field and Laboratory Analyses

The environmental variables were investigated between August 11 and September 18, 1982. The methods used to estimate lake morphometry variables, watershed area and the morphoedaphic index (MEI: Ryder et al. 1974) were fully described in Pinel-Alloul et al. (1990). Water sampling and conservation were performed following procedures established by Environment Canada (1979). All analytical chemistry methods were made according to Bobée et al. (1982) and described by Pinel-Alloul et al. (1990). Alkalinity determinations were made by the Gran titration method (Kramer 1980). Alkalinity/sulphate ratio was also computed to evaluate the importance of the substitution of bicarbonate ions by sulphate ions within surface waters. This ratio indicates the degree of "anthropogenic acidification" (Bobée et al. 1982).

Phytoplankton was sampled for a period of 1 mo (July 9 to August 10, 1982). In order to minimize the effect of the seasonal population development on the phytoplankton estimates, sampling was started at successive times in the eight areas (Fig. 1) following the west to east geographic axis. Composite water samples were collected by dipping a 2.4-cm diameter, 5-m long, flexible PVC tube in epilimnetic waters (0–5 m). Acidified Lugol's iodine solution was added immediately to preserve the phytoplankton samples for quantitative analyses. In the laboratory, samples were concentrated to 100 mL by sedimentation during a week period. Thereafter, 5-mL aliquots of each concentrated sample were settled in a sedimentation chamber for a 24-h period. Phytoplankton species were counted with a magnification of 600× on an inverted microscope using the Utermohl method (Lund et al. 1958). Taxonomic identifications were made by Desilets (1984) and based on keys in Smith (1950); Prescott (1962); Findlay and Kling (1979); and Patrick and Reimer (1966). Taxa were categorized by groups (chlorophytes, cyanophytes, chrysophytes, pyrrophytes) and species, except for the microflagellates which consist of unidentified, very minute (size <5 μm) and flagellate taxa. A minimum of 100 algal counting units (cells, filaments, and colonies) were counted per sample, which gave a precision of ±20% on total algal cell numbers. Counts were expressed as cells per millilitre.

Statistical Methods

The statistical approach used here to analyse the data involved a series of principal component, cluster, factor, and correlation analyses that were performed at the Université de Montréal using SPSS (Nie et al. 1975; Hull and Nie 1981) and R (Legendre and Vaudor 1984) packages of computer routines.

The phycological community structure was first studied using the abundances of phytoplankton groups (microflagellates, chlorophytes, cyanophytes, chrysophytes, pyrrophytes). This phycological data set was transformed using Box-Cox-Bartlett analysis (Sokal and Rohlf 1981) in order to normalize the distribution of the variables and to achieve homoscedasticity. This transformed data matrix was then partitioned into clusters of lakes by means of a flexible linkage cluster analysis (Lance and Williams 1966, 1967) with a β value fixed at -0.25 in order to minimize the chaining phenomenon (Sneath and Sokal 1973). We used the Gower similarity coefficient which excludes double zeros (not considered as a similarity: Legendre and Legendre 1979). In an attempt to obtain an ordination of the lakes based on the relative importance of phytoplankton groups, a principal component analysis was also performed on the correlation data matrix.

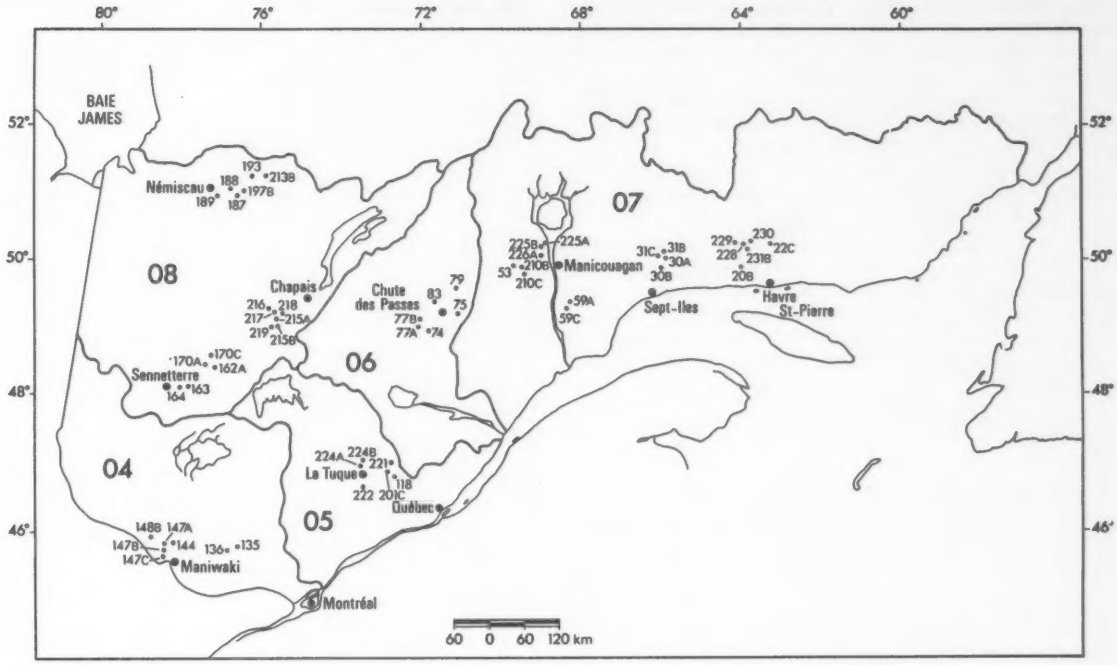
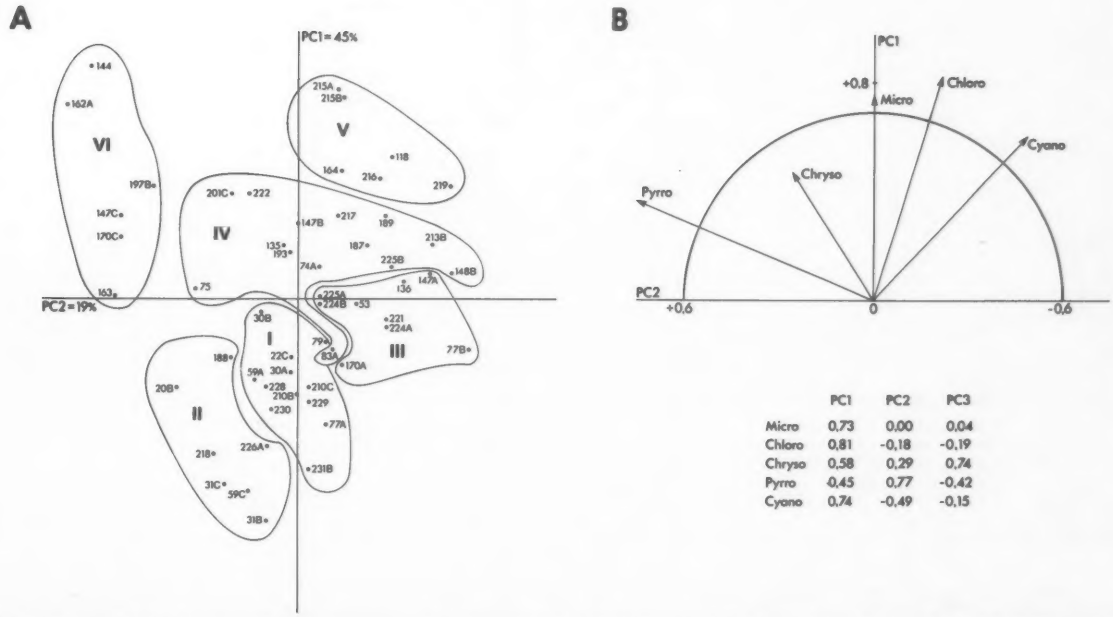


FIG. 1. Location of lakes sampled in Quebec.



	PC1	PC2	PC3
Micro	0,73	0,00	0,04
Chloro	0,81	-0,18	-0,19
Chryso	0,58	0,29	0,74
Pyrro	-0,45	0,77	-0,42
Cyano	0,74	-0,49	-0,15

FIG. 2. Clusters of lakes based on phytoplankton groups abundances (A). Principal component analysis and contribution of phytoplankton groups to the first three components (B). MICRO-microflagellates, CHLORO-chlorophytes, CYANO-cyanophytes, CHRYSO-chrysophytes, PYRRO-pyrrophytes. Contributions to PCA axes.

Otherwise, morphometric, physical, and chemical data were also transformed and a factor analysis was performed in order to simplify the variance structure of these highly correlated and interdependent variables (Pinel-Alloul et al. 1990). This process results in orthogonally rotated (varimax) factor scores, each factor representing an integrated environmental property correlated to a set of colinear abiotic variables.

Finally, this factor analysis served to simplify the analysis of relationships between abiotic factors and phytoplankton variables. The factor scores were correlated with the abundances of total phytoplankton, groups and species, using Spearman correlations. Furthermore, the use of Spearman rank correlations of phytoplankton species abundances with surface water temperature and dissolved oxygen at time of phytoplankton sampling also allowed us to verify the effect of these seasonal variables on the spatial variation of phytoplankton species estimates.

Results

Spatial Variation in Phytoplankton Groups Abundance

The first and second axes of the PCA account for 65% of the total variance in the abundances of phytoplankton groups (Fig. 2A). The first axis (45%) represents an ascending gradient of abundance in the chlorophytes, the cyanophytes, and the microflagellates (Fig. 2B). The second (19%) and the third axis (16%) are positively related to the pyrrophytes and chrysophytes abundances, respectively (Fig. 2B).

Based on the dominance patterns, the lakes can be partitioned into six clusters (Fig. 2A.). The first axis (PC1) distinguishes groups I and II (negative loadings) from group V (positive loading). The second axis (PC2) differentiates lakes of group VI (positive loading) from lakes of groups III, IV, and V (negative loadings).

Total phytoplankton mean density in lakes ranged between 690 to 9163 cells · mL^{-1} (Table 1). Lakes from group I are located in Eastern Quebec (Havre St-Pierre, Chute des Passes, Sept-Îles and Manicouagan areas). These lakes have the lowest total phytoplankton abundances (1128 cells · mL^{-1}). Lakes from group II are also mostly located in the Eastern region (mainly in the Manicouagan and Sept-Îles areas). Their total phytoplankton mean density is also low with 1607 cells · mL^{-1}. Phytoplankton communities of these two groups of lakes (I and II) are mainly composed of microflagellate algae (70–92%) which are represented by cryptophytes or chrysophytes taxa (Désilets 1984). Lakes from group V are located in western Quebec (mostly from Chapais area). Their mean total phytoplankton density is the highest (7935 cells · mL^{-1}). The microflagellates and the cyanophytes are co-dominant groups in their phytoplankton communities. Lakes from groups III and IV have intermediate positions and total phytoplankton mean densities ranging from 2762 to 3982 cells · mL^{-1}. They differ from each other in the greater relative importance of the cyanophytes in group III lakes than in group IV lakes. Most of the lakes from group VI are situated in the most western regions of Quebec (Senneterre and Maniwaki). Their phytoplankton communities can be characterized by greater abundances of

TABLE 1. Geographical area, lake number, mean ($\bar{X}$), standard deviation (SD) and extremes (min, max) of the abundances of total phytoplankton and groups for clusters of lakes determined by a flexible linkage cluster analysis (see Fig. 2 for legends).

Clusters	Areas	Lake number		*N* total	Micro	Chloro	Chryso	Pyrro	Cyano
I	Chute des Passes	77A, 79	$\bar{X}$	1128	797	126	113	0.5	92
	Manicouagan	59A, 210C	SD	376	318	172	70	0.7	60
	Havre St-Pierre	230, 231B, 228, 229, 22C	min	690	416	14	13	0	43
	Sept-Iles	30A, 30B	max	1760	1520	606	234	2.2	190
II	Sept-Iles	31C, 31B	$\bar{X}$	1607	1474	30	84	0.4	19
	Manicouagan	59C, 210B, 226A	SD	504	474	51	86	0.5	8
	Chapais	218	min	899	794	0	32	0	9
	Havre St-Pierre	20B	max	2537	2345	139	289	1	33
	Nemiscau	188							
III	La Tuque	224B, 2231, 224A	$\bar{X}$	2762	1134	124	80	0.6	1423
	Manicouagan	225A, 53	SD	926	223	89	55	0.9	833
	Maniwaki	147A, 136	min	1828	746	15	8	0	513
	Senneterre	170A	max	4050	1452	315	177	2.6	2483
	Chute des Passes	77B							
IV	Chute des Passes	75, 83A, 74A	$\bar{X}$	3982	2949	238	209	1.1	585
	La Tuque	201C, 222	SD	919	736	163	286	1.4	259
	Némiscau	193, 189, 187, 213B	min	2571	1782	3	13	0	156
	Chapais	217	max	5446	4125	493	1097	3.9	1010
	Manicouagan	225B							
	Maniwaki	148B, 135, 147B							
V	Chapais	215A, 215B, 216, 219	$\bar{X}$	7935	3309	828	417	0.7	3381
	La Tuque	118	SD	1212	1140	718	247	0.8	1181
	Senneterre	164	min	5863	2280	266	113	0	2075
			max	9163	5333	2035	734	2	4948
VI	Senneterre	170C, 163, 162A	$\bar{X}$	3796	2628	441	266	19.4	441
	Maniwaki	147C, 144	SD	1983	1234	381	191	12.0	488
	Némiscau	197B	min	2122	1351	85	46	10.9	44
			max	7263	4803	985	571	40.7	1415

TABLE 2. Mean ($\bar{X}$) and standard deviation (SD) of the physical, chemical, and morphometric variables for clusters of lakes determined by a flexible linkage cluster analysis. (See Table 1 for lake number in each cluster). * = Kruskall-Wallis ANOVA test significance. ns = $\alpha > 0.05$.

Clusters of lake		H^{+a*} ($\mu eq \cdot L^{-1}$)	Ca^{+b} ($\mu eq \cdot L^{-1}$)	Mg^{+c} ($\mu eq \cdot L^{-1}$)	K^{+b} ($\mu eq \cdot L^{-1}$)	Na^{+b} ($\mu eq \cdot L^{-1}$)	SO_4^{c} ($\mu eq \cdot L^{-1}$)	Alk[a] ($\mu eq \cdot L^{-1}$)	Trans[a] ($\mu eq \cdot L^{-1}$)
	$\bar{X}$	1.6	49.3	16.5	4.5	19.1	45.5	28.6	4.5
I	SD	2.4	17.6	7.4	2.9	4.2	12.6	26.3	1.3
	$\bar{X}$	1.6	52.8	26.6	3.4	20.6	52.1	38.2	3.1
II	SD	1.4	37.3	27.3	0.9	5.0	11.1	59.7	1.0
	$\bar{X}$	0.7	151.3	38.8	7.4	27.8	94.9	116.9	4.6
III	SD	1.2	159.7	18.7	3.0	9.5	43.1	164.4	1.8
	$\bar{X}$	0.5	121.0	41.6	8.4	26.1	87.8	85.7	3.4
IV	SD	0.5	131.2	29.1	3.3	7.1	38.5	126.6	1.6
	$\bar{X}$	0.2	206.7	126.5	8.1	25.6	72.9	280.2	2.7
V	SD	0.3	105.2	81.8	2.5	2.4	17.4	203.4	0.8
	$\bar{X}$	0.8	253.3	43.7	8.1	28.8	114.5	208.2	3.5
VI	SD	0.9	303.3	33.1	4.9	2.5	68.1	290.9	1.8

		Mn^{++} ns ($\mu g \cdot L^{-1}$)	TOC ns ($mg \cdot L^{-1}$	DOC ns ($mg \cdot L^{-1}$	Cond[c] ($\mu S \cdot cm^{-1}$)	Al[a] ($\mu g \cdot L^{-1}$)	Iron[a] ($\mu \cdot L^{-1}$)	pH[a]	Color ns U.H.	Alk/SO_4 ns ratio
	$\bar{X}$	9.8	5.2	5.2	11.8	140.3	62.1	6.1	33.9	0.5
I	SD	8.2	1.8	1.7	3.4	43.4	43.6	0.5	19.4	0.4
	$\bar{X}$	13.1	6.4	6.5	12.5	145.4	124.2	6.1	48.7	0.8
II	SD	3.8	2.5	2.4	3.2	70.3	55.6	0.6	28.1	1.5
	$\bar{X}$	9.6	5.9	5.3	33.6	71.8	55.2	6.7	27.4	1.2
III	SD	7.7	1.7	1.5	38.9	52.8	48.7	0.8	28.0	1.3
	$\bar{X}$	12.7	7.0	6.6	20.2	96.7	128.2	6.6	40.7	0.9
IV	SD	10.0	2.5	2.2	9.5	57.3	108.3	0.6	28.8	1.0
	$\bar{X}$	14.8	5.5	5.7	35.2	75.0	134.2	7.0	37.5	4.4
V	SD	3.7	1.5	2.2	15.7	28.0	69.0	0.6	18.1	3.4
	$\bar{X}$	11.7	6.5	6.3	29.8	81.0	86.5	6.7	31.7	1.2
VI	SD	7.9	1.9	2.2	23.0	70.3	82.0	1.1	30.4	1.5

		Max depth[a] (m)	Mean depth[d] (m)	Lake area ns (ha)	Watershed area[a] (ha)
	$\bar{X}$	34.6	7.7	261.4	7715.3
I	SD	24.9	4.0	112.9	7361.5
	$\bar{X}$	25.4	5.7	201.7	4921.4
II	SD	21.2	4.0	127.3	5736.2
	$\bar{X}$	17.7	4.1	146.8	2581.0
III	SD	13.7	2.2	8.4	2772.7
	$\bar{X}$	16.6	5.0	198.9	2168.9
IV	SD	11.5	3.8	188.3	3469.6
	$\bar{X}$	9.0	1.9	240.0	1550.8
V	SD	3.5	0.5	90.5	376.4
	$\bar{X}$	18.3	4.8	203.3	2483.3
VI	SD	23.5	5.6	134.1	3418.5

[a] $\alpha \leq 0.05$.
[b] $\alpha \leq 0.001$.
[c] $\alpha \leq 0.0001$.
[d] $\alpha \leq 0.01$.

pyrrophytes than those of lakes of other groups. Kruskall-Wallis ANOVA (Siegel 1956) of the differences between clusters of lakes in the abundance or the percentage of each phytoplankton group indicates significant spatial variation for all groups ($\alpha < 0{,}01$) except for the chrysophytes, when expressed in percentages. Spatial heterogeneities between clusters of lakes were also noted (Kruskall-Wallis tests; $\alpha < 0{,}05$) for the abiotic variables (Table 2), but managanese, total and dissolved organic carbon, color, alkalinity/sulphates ratio, and lake area. Lakes from groups I and II have the most acidic and soft waters. They receive low sulphates deposition and have the highest total aluminum concentrations; their alkalinity/sulphates ratio is lower than 1. These groups correspond to naturally dilute and deep lakes with generally large watershed areas, which are very sensitive to acidification. In contrast, lakes from group V and VI are alkaline and hardwater lakes. Group VI represents lakes with the greatest sulphates deposition. Group V is characteristic of shallow lakes not subjected to important sulphate atmospheric deposition; these lakes show high iron concentration and low water transparency. Group III and IV are circumneutral lakes, very susceptible to acidification as indicated by high amount of sulphates and alkalinity/sulphates ratio lower than 1 in group IV.

Environmental Factor Scores

The factor analysis on abiotic variables, already presented in the zooplankton study of the same lake sample (Pinel-Alloul et al. 1990), shows that 73% of the total variance is explained by five derived environmental factors (Table 3). Factor 1

TABLE 3. Spearman rank correlation coefficients between total phytoplankton, groups and taxa abundances, and the first five environmental factors and seasonal factors. (significance = *$0.05 \geq \alpha > 0.01$; **$0.01 \geq \alpha > 0.001$; ***$\alpha \leq 0.001$), T° = water temperature, DO = dissolved oxygen at time of phytoplankton sampling.

Phytoplankton variables	Percent lake occurrence	Factor 1 hardness–alkalinity	Factor 2 lake depth transparency	Factor 3 dystrophy	Factor 4 lake size	Factor 5 salinity sulphates	Seasonal factors	
							T°	DO
Total phytoplankton		0.36**	−0.46***	—	—	0.37**		
Micro flagellates		—	−0.39**	—	—	0.30*		
Chlorophytes		0.38**	−0.25*	—	—	0.33**		
Selenastrum minutum	40	0.22*	—	—	—	0.27*	—	—
Ankistrodismus falcatus	23	—	—	—	—	—	—	0.32*
Oocystis sp.	37	—	—	—	−0.25*	—	—	—
Oocystis pulsilla	6	0.24*	—	—	—	—	—	—
Dictyosphaerium ehrenbergianum	19	—	—	0.28*	—	—	−0.28*	—
Gleocystis sp.	24	0.32*	—	−0.23*	—	0.54***	0.57***	−0.32*
Crucigenia tetrapedia	16	0.44***	—	—	—	—	0.45**	−0.26*
Tetraedron minimum	23	0.44***	—	—	—	—	—	—
Mougeotia sp.	6	—	—	—	—	—	−0.25*	—
Crucigenia sp.	7	—	0.26*	—	—	—	−0.25*	—
Pediastrum tetras	6	—	—	—	—	—	—	—
Arthrodesmus incus	23	—	−0.23*	—	—	—	—	—
Scenedesmus sp.	8	—	—	—	—	0.28*	—	—
Elakatothrix gelatinosa	17	—	—	—	—	—	0.31*	−0.34**
Closteriopsis longissima	6	—	—	—	0.27*	—	—	—
Cosmarium sp.	18	0.42**	—	—	—	—	0.30*	—
Quadrigula sp.	25	—	—	0.29*	—	0.35**	—	—
Botryococcus sudeticus	7	—	—	—	—	0.34**	0.27*	—
Botryococcus braunii	7	—	—	—	—	—	—	—
Pyrrophytes		—	—	—	—	0.26*		
Peridinum sp.	20	—	—	—	—	—	—	—
Peridinium inconspicuum	11	—	—	−0.24*	—	—	0.24*	—
Chrysophytes		0.25*	—	—	—	—		
Kephyrion sp.	7	−0.43***	—	—	—	—	—	0.30*
Mallomonas sp.	37	−0.23*	—	—	—	—	—	—
Desmarella sp.	8	0.23*	—	—	—	0.25*	—	—
Bitrichia chodatii	41	—	—	—	−0.27*	—	—	—
Chrysolykos skujae	5	—	0.40**	—	0.27*	−0.24*	−0.28*	0.23*
Dinobryon crenulatum	8	—	0.49***	—	—	—	—	—
Chrysosphaerella longispina	7	—	—	—	—	0.25*	—	—
Dinobryon sertularia	17	—	−0.29*	—	—	—	—	—
Rhizosolenia eriensis	22	—	—	—	0.27*	—	—	0.26*
Dinobryon bavaricum	41	—	—	—	—	—	—	0.31*
Dinobryon sp.	20	—	—	—	—	—	−0.28*	—
Asterionella formosa	36	—	−0.37**	—	—	—	—	—
Tabellaria flocculosa	18	—	—	—	—	—	—	—
Tabellaria fenestrata	35	—	−0.29*	—	—	0.40**	—	—
Synedra sp.	5	—	−0.27*	—	—	—	—	—
Synedra acus	5	—	—	—	—	—	—	—
Synedra nana	16	0.49***	—	—	—	—	0.29*	—
Synedra ulna	7	—	0.29*	—	—	—	0.27*	—
Eunotia sp.	7	0.39**	—	—	—	—	—	—
Navicula sp.	18	—	—	—	—	−0.31*	—	—
Stephanodiscus sp.	8	—	—	—	0.31*	—	—	—
Nitzchia sp.	13	—	—	0.28*	—	—	—	—
Melosira (Aulacoscira) italica	14	—	−0.35*	0.35**	—	—	—	—
Cyclotella sp.	20	0.44***	—	—	—	—	—	—
Cyanophytes		0.48***	−0.35**	—	−0.24*	0.32**		
Anabaena flos-aquae	9	0.38**	—	—	—	—	0.32*	—
Oscillatoria limnetica	11	0.40**	—	—	—	—	0.28*	—
Aphanocapsa delicatissima	25	0.41**	—	—	—	0.36**	0.40**	—
Anabaena sp.	9	0.38**	—	—	—	—	—	—
Coelosphaerium sp.	9	0.29*	−0.32*	—	—	—	—	—
Lyngbya limnetica	5	0.48***	−0.37**	—	—	—	—	—
Aphanothece clathrata	10	—	—	—	—	—	—	0.29*
Merismopedia tenuissima	29	—	—	—	—	—	—	—
Chroococcus limneticus	17	0.27*	—	—	—	0.59***	0.54***	−0.26*
Chroococcus dispersus	36	0.28*	—	—	—	0.34**	0.39**	—
Dactylococcopsis smithii	6	0.28*	—	—	—	—	0.33*	—
Aphanocapsca elachista	26	0.56***	—	—	—	0.39**	0.47***	—
Aphanothece nidulans	50	0.61***	−0.29*	—	—	—	0.34**	—
Microcystis sp.	10	0.30*	—	—	—	—	0.30*	−0.39**
Chroococcus sp.	10	—	—	0.28*	—	—	—	—
Aphanocapsa sp.	11	—	—	—	—	—	—	—
Merismopedia minima	29	—	−0.37**	—	—	—	—	—
Gomphosphaeria sp.	5	0.33**	—	—	—	—	—	—
Dactylococcopsis acicularis	36	0.39**	—	—	—	—	—	—

explains 21% of the total variance and is considered a hardness-alkalinity factor. Factor 2, which explains 17% of the total variance, corresponds to the lake depth and transparency factor. Factor 3 responsible for 14% of the total variance, represents the dystrophy factor. Factor 4 explains 11% of the total variance and is the lake size factor. Factor 5 responsible for 10% of the total variance, indicates the salinity-sulphates factor.

Phytoplankton Relationships with Environmental Factors Scores

One hundred twenty-nine phytoplankton taxa were identified from the 54 lakes examined in this study. An average of 24 taxa were found per sample ranging between 8 and 43. Table 3 shows correlations between the abundances of phytoplankton groups and dominant taxa and the five derived environmental factors. Species or taxa present in less than 10% of the samples sites (≤5 lakes) were not retained for this analysis. Total phytoplankton, cyanophytes, and chlorophytes abundances were positively correlated with the hardness-alkalinity and salinity-sulphates factors and negatively correlated with the lake depth and transparency factor. Microflagellates are more abundant in lakes with high salinity-sulphates loading and less abundant in deep and clear lakes. However, when based on relative abundances, relationships observed for the microflagellates were inversed. The chrysophytes show few significant relationships with the environmental factors scores, but factor 1. Positive relationships were noted for the pyrrophytes with the salinity-sulphates factor.

Within each phytoplankton group, a large number of taxa were correlated with factor 1 (alkalinity-hardness). Species with the highest significant correlation coefficients are mainly cyanophytes (*Aphanothece nidulans, Aphanocapsa elachista, Lyngbya limnetica, Aphanocapsa delicatissima, Oscillatoria limnetica, Anabaena flos-aquae, Dactylococcopsis acicularis, Anabaena* sp.), diatoms (*Synedra nana, Cyclotella* sp., *Eunotia* sp.), and chlorophytes (*Crucigenia tetrapedia, Tetraedron minimum, Cosmarium* sp.). There are only two chrysophyte taxa showing significant negative correlation with factor 1: *Kephyrion* sp. and *Mallomonas* sp.

Only a small number of taxa are positively correlated with factor 2 (lake depth and water transparency), especially the chrysophytes *Dinobryon crenulatum* and *Chrysolykos skujae*. A greater number of species are negatively correlated with factor 2, especially the chrysophycean and diatom species (*Asterionella formosa, Melosira* (*Aulacoscira*) *italica, Tabellaria fenestrata, Dinobryon sertularia, Synedra* sp.) and the cyanophytes (*Lyngbya limnetica, Merismopedia minima, Coelosphaerium* sp., *Aphanothece nidulans*).

A few taxa, including *Dictyosphaerium ehrenbergianum, Quadrigula* sp., *Nitzchia* sp., *Melosira (Aulacoscira) italica* and *Chroococcus* sp. are positively correlated with factor 3 (dystrophy). *Peridinium inconspicuum* and *Gloeocystis* sp. are negatively associated with factor 3.

Factor 4 (lake size) also shows significant correlations with few species. Positive correlations are noted with *Stephanodiscus* sp., *Closteriopsis longissima, Chrysolykos skujae*, and *Rhizosolenia eriensis*. Only, *Oocystis* sp. and *Bitrichia chodati* present negative correlations with the lake size factor.

Many species, especially among cyanophytes and chlorophytes (*Chroococcus limneticus, Gloecystis* sp., *Aphanocapsa elachista, Aphanocapsa delicatissima, Chroococcus dispersus, Quadrigula* sp., *Botryococcus sudeticus*) and some diatoms (*Tabellaria fenestrata*) present positive correlations with factor 5 (salinity-sulphates loading). As a group, the pyrrophytes also are positively linked to the factor 5. Negative correlations with factor 5 are noted for *Navicula* sp. and *Chrysolykos skujae*.

Some species are significantly correlated with one or both seasonality factors. The warm water species are among the cyanophytes (*Chroococcus limneticus, Aphanocapsa elachista, Aphanocapsa delicatissima, Chroococcus dispersus, Aphanothece nidulans, Dactylococcopsis smithii, Anabaena flos-aquae, Microcystis* sp., *Oscillatoria limnetica*), the chlorophytes (*Gleocystis* sp., *Crucigenia tetrapedia, Elakatothrix gelatinosa, Cosmarium* sp., *Botryococcus sudeticus*) and the chrysophytes (*Synedra nana, Synedra ulna*). Cold water species consist mainly of chrysophytes (*Chrysolykos skujae, Dinobryon* spp.) and chlorophytes (*Dictyosphaerium ehrenbergianum, Mougeotia* sp., *Crucigenia* sp.). Less significant relationships are observed with dissolved oxygen. Some cyanophytes (*Microcystis* sp., *Chroococcus limneticus*) and chlorophytes species (*Elakatothrix gelatinosa Gleocystis* sp., *Crucigenia tetrapedia*) are generally associated with low oxygenated waters while chrysophytes species (*Dinobryon bavaricum, Kephyrion* sp., *Rhizosolenia eriensis, Chrysolykos skujae*), the chlorophytes *Ankistrodesmus falcatus* and the cyanophytes *Aphanothece clathrata* are linked to well oxygenated waters.

Discussion

Since some methodological problems (fixed sampling depth, two few acid lakes, lack of TP measurements) related to the sampling design established and carried out by Department of Fisheries and Oceans Canada could have limit the scope of our study, cluster analysis and correlations between phytoplankton taxa and the five synthesized factors should be interpreted with care. In order to be confident in ecological interpretations based on phytoplankton distribution in our study, it is necessary to demonstrate similar conclusions from other regions. This study will thus be compared with phytoplankton associations of insular Newfoundland and Labrador lakes.

First, comparisons of the morphometry and water chemistry in our lake sample with other lake survey monitored in the six provinces of Eastern Canada (Kelso et al. 1986; Scruton 1983, 1984; Lachance et al. 1984) allows us to consider our lake sample as representative of eastern Canada lakes with little bias toward higher alkalinity and pH, and lower levels of organic carbon and sulphates (Pinel-Alloul et al. 1990). In Quebec, the general abiotic features of lakes can be characterised by five integrated factors: alkalinity-hardness, lake depth and transparency, dystrophy, lake-size, and salinity-sulphates loading. In Newfoundland and Labrador lakes, the most important factors were similar: dystrophy, hardness, lake size, and salinity (Earle et al. 1986, 1987). In Newfoundland, dystrophy, hardness, and salinity accounted for most of the variability (71%) in physico-chemical properties between lakes (Earle et al. 1987). Hardness, dystrophy, and lake size explained also a great proportion of the variability (62%) between lakes in Labrador (Earle et al. 1986). In Quebec lakes, the hardness-alkalinity, lake depth and transparency, and dystrophy factors are the first three important environmental factors, but the variance explained by these factors (52%) is slightly smaller than the variance explained by the first three factors in Newfoundland and Labrador studies. This may be explained by the absence in our study of important explanatory parameters such as phos-

phorous which was not measured. Nevertheless, phosphorus does not seem important in Labrador and Newfoundland studies (Earle et al. 1986, 1987). The major cause must also be a greater similarity in physicochemical and morphometric characteristics within our studied 54 Quebec lakes. In Labrador and in Newfoundland lakes, pH is related positively to hardness and negatively to dystrophy. In Quebec lakes, this variable is only positively related to the hardness-alkalinity factor.

Three major geographic areas are distinct according to the abundances and dominance of phytoplankton groups. Northeastern lakes (groups I and II: Sept-Îles, Havre St-Pierre, Manicouagan) support poor phytoplankton communities dominanted by the microflagellates group. Middle-west lakes (groups III, IV, and VI: Chute des Passes, Senneterre, Maniwaki, Némiscau, La Tuque) have averaged phytoplankton density. They differ from each other by variations in the relative importance of the cyanophytes or the pyrrophytes. Finally, the western lakes (group V: Chapais) have the highest phytoplankton density and are co-dominated by the cyanophytes and the microflagellates. There is an increase in total phytoplankton and groups abundances related to the longitudinal east–west axis. The same pattern was observed for zooplankton populations (Pinel-Alloul et al. 1987, 1990). However, inside each area there are important variations because not all the lakes of the same region are grouped in the same cluster (Table 1). Some aspects of the design sampling, especially the fixed sampling depth (0–5 m), could result in an underestimation of phytoplankton density in the most clear and dilute lakes of the northeastern regions of Quebec. Kettle et al. (1987) showed that metalimnetic phytoplankton peaks occurred in a small acidified and oligotrophic lake of the Precambrian Shield, but no systematic study of this phenomenon has been realized on a large sample of acidified lakes. It is thus difficult to estimate with precision the biais introduced by the sampling procedure in our study. Nevertheless, we are confident in the geographic density gradient observed in phytoplankton of Quebec lakes since in a similar study on phytoplankton community in 50 lakes located in Maniwaki, Sept-Îles, Gagnon, and Schefferville areas, Pope et al. (1985) also found lower density in lakes from Sept-Îles and Gagnon areas ($0.1–0.4 \times 10^6$ cells $\cdot L^{-1}$) than in lakes from Maniwaki area ($0.6–4.4 \times 10^6$ cells $\cdot L^{-1}$). They also observed a strong variability between lakes of a same area mainly due to pH differences and, they also noted the same geographic ascending gradient from east to west for the cyanophytes and the chlorophytes.

Characteristic features of phytoplankton structure in acidified or alkaline lakes have been well documented but, as said by Stokes (1986), no clear pattern has yet emerged. In most cases, diatoms, chrysophytes, and euglenophytes are rare or absent in acidified waters while pyrrophytes, cyanophytes, and chlorophytes dominante (Johnson et al. 1970; Findlay and Seasura 1980; Stoneburner and Smock 1980; Havens and DeCosta 1986); Kwiatkowski and Roff (1976) noted an increase in cyanophytes and a decrease in chlorophytes abundances with lake acidification. Brezonik et al. (1984) detected the opposite trends in Florida lakes. Yan (1979); Erikson et al. (1980); and Haines (1981) have observed a replacement of chlorophytes and cyanophytes by pyrrophytes in the most acidic lakes. However, in contrast, Brock in Muller (1980) and Blouin et al. (1984a, b) observed a decrease of cyanophytes in acidified lakes while Wright et al. (1976) have noticed a diminution of chlorophytes in relation with a decrease of pH.

Alkalinity–acidity gradient within Quebec lakes is the most important factor related to phytoplankton distribution. Total phytoplankton and relative abundances of groups (particularly the cyanophytes and the chlorophytes) and higher in hardwater and alkaline lakes with high sulphate loadings (Table 3). Species presenting the highest positive correlations with the hardness-alkalinity factor either in Quebec or in Newfoundland and Labrador are cyanophytes (*Aphanocapsa elachista, Aphanothece nidulans, Lyngbya limnetica, Aphanocapsa delicatissima, Anabaena* sp., *Anabaena flos-aquae, Oscillatoria limnetica, Dactylococcopsis acicularis*), chlorophytes (*Crucigenia tetrapedia, Tetraedron minimum, Cosmarium* sp.) and some diatoms (*Synedra nana, Cyclotella* sp. *Eunotia* sp.). According to the distribution functions of phytoplankton species with nutrient supply defined by Rosen (1981), all those cyanophytes and chlorophytes are eutrophic indicators. The chrysophycean species (*Kephryon* sp., *Mallomonas* sp.) negatively correlated to factor 1, present similar relationships in Newfoundland and Labrador lakes. *Mallomonas* spp. has been reported as a dominant taxon below pH 5.6 in experimental lake acidifcation (Findlay and Kasian 1986) and *Kephryon* sp. is related to oligotrophy (Earle et al. 1986). Cyanophytes distribution in Quebec lakes seem to differ from the distribution reported by Kwiatkowski and Roff (1976) on six acidified lakes (pH: 4.38–6.75) in the Sudbury region (Ontario) where the most acidic lakes (pH<5) were all dominated by blue-green algae (>58%). However, predominant cyanophytes in the less acidic lakes (*Chrooccocus dispersus, Chroococcus limneticus, Aphanocapsa* spp., and *Oscillatoria* sp.) were similar to the abundant cyanophytes species reported in alkaline lakes of western Quebec.

Effects of anthropogenic acidification on phytoplankton distribution, as expressed by the relationships with factor 5 (salinity-sulphates loading), are difficult to interpret since sulphates are also positively related to the hardness-alkalinity factor and inversely related to the lake size factor. In Labrador lakes, sulphates were related to the hardness and dystrophy factors (Earle et al. 1986) and, in Newfoundland lakes, they were related to the dystrophy and salinity factors (Earle et al. 1987). Most of the species indicators of anthropogenic acidification were cyanophytes and chlorophytes taxa (*Chrooccocus limneticus, Gloeocystis* sp., *Aphanocapsa elachista, Aphanocapsa delicatissima, Chroococcus dispersus, Bothryococcus, sudeticus*, and *Quadrigula* sp.) which also were generally positively related to factor 1. These species were found dominant in Sudbury acidified lakes by Kwiatkowski and Roff (1976). Among the chrysophytes, *Tabellaria fenestrata* showed greater abundances in lakes subjected to sulphates deposition but were not correlated to the hardness-alkalinity factor. This colonial diatom is reported to live in low calcium and neutral-acidic waters (Round 1973; Kwiatkowski and Roff 1976) and it is considered as an oligomesotrophic (Rosen 1981), acid-tolerant (Havens and DeCosta 1986) species indifferent to salinity (Beaver 1981).

Morphological features of lakes (factors 2 and 4) also influence the phytoplankton distribution but at a lesser extent than the alkalinity-acidity gradient. All phytoplankters groups are less abundant in clear and deep lakes of eastern Quebec where the phytoplankton community is dominated by microflagellates. This unspecified group represents mainly small chrysophytes and cryptophytes taxa which predominate in oligotrophic waters (Rosen 1981). The few species positively related to the lake depth and transparency factor are mainly chrysophytes (*Dinobryon crenulatum, Chrysolykos skujae*) and chlorophytes

(*Crucigenia* sp.). *Dinobryon crenulatum*, not recorded in Labrador lakes (Earle et al. 1986) is considered an indicator of oligotrophy (Rosen 1981). It was inversely related to hardness and dystrophy factors within Newfoundland lakes (Earle et al. 1987). *Chrysolykos skujae* is correlated both to lake size and hardness factors in Labrador lakes. Among chrysophycean species, *Rhyzosolenia eriensis* does not present a correlation with the factor 2 but with the lake size factor (factor 4) as in Newfoundland lakes (Earle et al. 1987) where it is also related to hardness and salinity factors. It is considered an oligotrophic–mesotrophic species (Rosen 1981) restricted to large lakes (Stoermer 1978). Most of the chlorophytes, cyanophytes, diatoms (*Asterionella formosa, Tabellaria fenestrata, Melosira (Aulacoscira) italica)* and chrysophytes (*Dinobryon sertularia*) were negatively related to factor 2 in Quebec lakes. The large diatoms presented inverse relationships in Labrador and Newfoundland lakes. Although *Bitrichia chodati* and *Oocystis* sp. have oligotrophic affinity (Rosen 1981), they show negative correlations with the lake-size factor.

Only a few species (*Melosira italica, Dictyosphaerium ehrenbergianum, Quadrigula* sp., *Nitzchia* sp., and *Chroococcus* sp.) seem to demonstrate a positive correlation with the dystrophy factor (factor 3). Unfortunately, most of these taxa were only identified to generic level. None of the species which were positively correlated with the dystrophy factor in Newfoundland and Labrador lakes (Earle et al. 1986, 1987) were correlated with that factor in Quebec lakes. Janus and Duthie (1979a) already noted that the distribution of diatoms in the Matamek region may be influenced by allochthonous organic matter. In Jarnefelt (1956) studies, chrysophycean plankton appeared more commonly distributed in humic waters. However, *Melosira (Aulacoscira) italica* is also found mostly in mesotrophic habitats over a wide range of pH (Beaver 1981; Del Prete and Schofield 1981). The chlorophytes *Dictyosphaerium ehrenbergianum* and *Quadrigula* sp. present affinites for mesotrophic or eutrophic waters generally with contributions of humic substances (Rosen 1981).

Our results are not consistent with those who related the distribution of pyrrophytes to acidity gradients (Bleiwas et al. 1984; Schindler et al. 1985). The abundance of pyrrophytes was higher in alkaline lakes subjected to sulphates deposition (factor 5) than in naturally dilute and slightly acidic lakes. Since we sampled too few low-pH (<5.5) lakes, our sample may not be representative of phytoplankton communities at all pH's. Although *Peridinium inconspicuum* is considered a species highly associated with acid lakes and a good indicator of acidification (Yan 1979; Findlay and Kasian 1986; Havens and DeCosta 1986) in oligotrophic or eutrophic lakes (Eloranta 1986; Kwiatkowski and Roff 1976), this species was not related to the natural acidity gradient in Quebec lakes where we detected only a negative relationship with dystrophy.

Our results do not show evidence of seasonal effect on phytoplankton estimates. Since sampling was restricted to 1-mo period, relationships with seasonality factors are probably a consequence of the longitudinal gradients in water temperature and oxygenation within the sampled lakes. This can be confirmed by the fact that most species strongly associated to the hardness-alkalinity factor present positive correlations with water temperature.

Despite methodological problems, this study contributes to the understanding of Quebec phytoplankton ecology. It shows that phytoplankton species distribution in relation to environmental factors associated to acidification, mineralisation, and lake morphometry corresponds well with phytoplankton species distributions described in insular Newfoundland and Labrador lakes (Earle et al. 1986, 1987). Environmental conditions represented by the five synthesized factors (alkalinity-acidity, lake depth and transparency, dystrophy, lake size, and sulphates loading) substantially influence phytoplankton species distribution in Quebec as in other regions in eastern Canada. Nevertheless, these factors fail to account fully for the total observed variance in phytoplankton distribution. Additional factors not considered in this study (trophic state, selective grazing, competition) are probably responsible for the residual heterogeneity.

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